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Ion transporting enzymes and their regulation by endogenous modulators

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Abstract

Ion transporting enzymes are responsible for the transport of ions across cell membranes. Depending on the nature of ions transported, they are named. Thus, when an enzyme transports Na^+ and K^+ ions, it is called Na^+, K^+ -ion transporting enzyme, when it is involved in the transport of Ca^{2+} ion, it is known as Ca^{2+} ion transporting enzyme and so on. Since the transport of ions take place against concentration gradient of ions, energy is required to transport them against the gradient, which is provided due to the catalysis of ATP to ADP and Pi. Since ATP is hydrolyzed during the transport of ions, the ion transporting enzymes are therefore called ATPases e.g. Na^+, K^+ -ATPase, H^+, K^+ -ATPase etc. As the transport of ions take place against concentration gradients and is energy dependent, this is an active process.

(**Keywords** : Na^+, K^+ -/Ca^+, Mg^{2+} -/Ca^{2+} -ATPase regulation; cationic amphiphilic drugs; modulator proteins)

Na^+, K^+ -ATPase

Sodium, potassium ion stimulated adenosine triphosphatase (Na^+, K^+ -ATPase; E.C. 3.6.1.3), commonly known as Na^+ -pump, first discovered by J.C. Skou in 1957 in crab nerve cell, is a membrane bound enzyme activated by Na^+ and K^+ ion[1]. Since then, several reports have appeared indicating its presence in different tissues. In red

blood cells, this process has been most extensively studied. The K^+ ion concentration in the cytosol is about 110 mM whereas in the blood plasma is only about 3 mM. On the other hand, the Na^+ ion concentration in the blood plasma is relatively high, about 140 mM, but in red blood cell is only about 4 mM. Maintenance of these gradients across the plasma membrane depends upon the input of ATP. The Na^+, K^+ -ATPase present in the red cell membrane catalyses the hydrolysis of ATP to ADP + Pi and the free energy released is used to pump $2K^+$ ion inward and $3Na^+$ ion outward. At the energizing step in this process, the terminal phosphate group of ATP is transferred to the Na^+, K^+ -ATPase molecule. This enzyme-bound phosphate group undergoes hydrolysis and appears as inorganic phosphate in the cytosol. The ADP and phosphate so formed are converted to ATP by the energy yielding breakdown of glucose.

A schematic diagram for the transport of Na^+ and K^+ ions across the membranes is shown in Fig. 1.

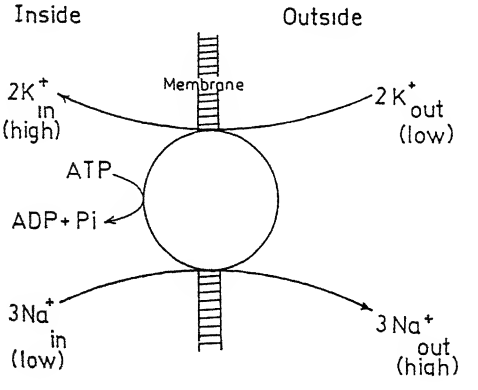


Fig. 1– Schematic diagram showing the transport of Na^+ and K^+ ions across cell membranes.

The Na^+, K^+ -ATPase is a member of the P-type class of ATPase that are characteristically phosphorylated and dephosphorylated like other P-type ATPases such as Ca^{2+} -ATPase and the H^+ K^+-ATPase of the sarcoplasmic reticulum and plasma membrane[2]. The overall Na^+, K^+ -ATPase activity is defined as the Na^+ and K^+ ion dependent increment of Mg^{2+}-ATPase activity which is generally expressed as the amount of Pi liberated from ATP by the enzyme per unit time at a particular temperature. It is specifically and characteristically identified by its inhibition by extracellular binding of cardiac glycosides, the most widely used and well known one is ouabain [3,4]. The description of this transport phenomenon proposed in the late 60's by Albers [5] and Post *et al*[6] that the enzyme exists in two different conformations, an E_1 and an E_2 conformation. According to Albers-Post reaction scheme, which consists

of four steps, the Na^+, K^+ -ATPase undergoes a transition between the E_1 conformation with inwardfacing cation binding sites and high affinity for Na^+ and the E_2 conformation with outward-facing cation binding sites and high affinity for K^+. Transitions between these two conformation are induced by phosphorylation-dephosphorylation reaction (scheme 1)

$$E + APT \underset{(1)}{\overset{Na^+(in)}{\rightleftharpoons}} E.ATP \underset{(2)}{\overset{ADP}{\rightleftharpoons}} E_1 \sim P \underset{(3)}{\overset{Mg^{2+}}{\rightleftharpoons}} E_2 \sim P \underset{(4)}{\overset{K^+(out)}{\rightleftharpoons}} E + Pi$$

Scheme 1

Step 1 of the reaction mechanism involves the binding of ATP in presence of Na^+ ion to the enzyme molecule. The Na^+ -form binds free ATP with high affinity, but K^+ -form binds it much less tightly (about 100 fold less)[7]. The 6-NH_2 group on the purine ring, the 2-OH group in the ribose and the β, γ - pyrophosphate group have been indicated as binding groups of the ATP molecule[8]. Binding sites on the enzyme appear to be an arginine guanidine group[9] which probably binds to the β, γ - pyrophosphate group of ATP. There is also report of the probable interaction of the tyrosyl-OH group and cysteinyl- SH group with the purine ring of ATP[10-12].

Step 2 represents a Mg^{2+} and Na^+ ion dependent phosphorylation of the enzyme, accompanied by breakdown of ATP to ADP. The resulting phosphorylated intermediate $E_1 \sim P$ is a high energy compound since it reacts with ADP and forms ATP[13]. $E_1 \sim P$ does not undergo dephosphorylation in the presence of K^+ ion [14-15].

Step 3 is believed to represent a conformational change of the enzyme molecule, from a Na^+ affinity state (E_1) to K^+ affinity state (E_2), during which the Na^+ ion binding sites are turned inside out[16]. The changed conformational state of the phosphorylated $E_2 \sim P$, is a low energy compound and does not react with ADP but decomposes in presence of K^+ ion; which indicates that $E_2 \sim P$ is an entity distinct from $E_1 \sim P$.

Step 4 involves K^+ ion stimulated dephosphorylation pathway during which the K^+ ion binding sites turn inward and the enzyme returns to its original conformation[17]. In consideration of the stereochemistry of the various interactions of the enzyme with substrate and activating cation, it has been shown that ATP and Mg^{2+} ion react on the inside of the membrane and that the hydrolysis products remain on this side[18].

The Na^+, K^+ -ATPase represents a universal machinery in the membranes of animal cells, which transfer chemical energy from the hydrolysis of ATP to potential energy of electrochemical ion gradients for Na^+ and K^+ ion across the cell membrane[16]. Na^+, K^+ -ATPase has been purified from the outer medulla of dog, sheep and rabbit kidneys[3,19-21]. The highest activity has been observed in excitatory and secretary tissues[22]. Other organs where the enzyme is enriched are being brain cortex, electrical electroplast[23], duck salt gland[24] and the enzyme is present mainly in the plasma membranes.

Purified Na^+, K^+ -ATPase is composed of at least two transmembrane non-convalently linked subunits, α and β[25]. The molecular mass of α-subunit ranges from 84-120 kDa and that of β-subunit from 40-60 kDa[19,23]. The smaller β subunit is a glycoprotein[26].

Dzhandzhugazyan *et al*[27] reported that in case of pig Na^+, K^+ -ATPase, molecular mass of α subunit is 112 kDa, β is ~ 44 kDa and the carbohydrate moiety is ~ 9 kDa and is linked by N-glycosidic bonds to the β subunit. Isoforms exist for both the α (α_1, α_2 and α_3) and β (β_1, β_2 and β_3) subunits[28,29]. The α_1 isoform occurs in most tissues, while the α_2 isoform is predominant in skeletal muscle and is also detected in the brain and the heart. The α_3 isoform is limited essentially to neural and cardiac tissue. The β_1 and β_2 subunits are the predominant isoforms in mammalian cells where β_1 is ubiquitously expressed and β_2 appears mostly in neural tissue. Thus far, the β_3 isoform has only been identified in *Xenopus*[30] The orientation of α and β subunits (as α_2 β_2 tetramer) of Na^+, K^+ -ATPase in the lipid bilayer is shown in Fig. 2. Both the subunits span whole lipid bilayer.

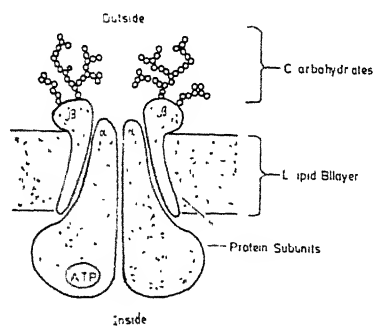


Fig. 2-The orientation of α and β subunits of Na^+, K^+ -ATPase in the lipid bilayer

An Artist's conception of the composition of Na^+, K^+ -ATPase (New England J. Med. 302 : 777-783, 1980. Reproduced with the permission of the Journal)

The individual catalytic roles(s) of each subunit is not well defined. α subunit comprises probably ten transmembrane helices is responsible for both catalytic and transport function[28,31,32].

The α subunit is considered to be an acceptor for the γ-phosphate of ATP. Phosphorylation of the aspartic acid residue (Asp-369)[33] leads to formation of the phosphorylated intermediate during the reaction cycle[34]. In addition to the cation binding sites, the receptor for cardiac glycosides is also located on the α subunit[35,36]. The α subunit Na^+, K^+ -ATPase also interacts with ankyrin utilizing residues 142-166 in its second cytoplasmic domain[37]. This interaction stabilizes Na^+, K^+ -ATPase at the plasma membrane and enables its transport from the endoplasmic reticulum to the golgi[38].

The β subunit is a type II membrane protein with a short cytoplasmic N-terminus, one transmembrane segment and an ectodomain that carries three disulfide bridges and several sugar chains. The β subunit appears to be involved in maturation of the enzyme, localization of the ATPase to the plasma membrane and stabilisation of a K^+ -bound intermediate form of the protein[39-44].

A third subunit called γ subunit with a molecular mass of about 12 kDa has also been identified and reported to be a tissue-specific regulator that stabilizes the E_2 conformation of the enzyme[45,46]. Recently, it has been demonstrated that the assembly of both the α and β subunits are necessary for functional expression of normal Na^+, K^+ -ATPase[47-49]. This individual α and β subunit leave to the endoplasmic reticulum and assemble at the plasma membrane[50]. A 26 amino acid sequence in an extracellular loop of the Na^+, K^+ -ATPase α subunit between membrane-spanning segments 7 and 8 has been shown to bind to the β subunit of Na^+, K^+ -ATPase and to promote αβ assembly[51]. Among these amino acids, Valine 904, Tyrosine 898 and Cysteine 908 in Na^+, K^+ -ATPase α subunit are important for assembly with β subunit[52]. Although several evidences supporting the stoichiometry of α and β is controversial, some reports predict it is 1 : 1[53-57] for the functional enzyme unit however according to another report it is 2 : 3[58]. Molecular mass of α and β subunits isolated from different sources are shown in Table 1.

Table 1– The best molecular weight estimates of the Na^+, K^+ -ATPase subunits from four species[a]

Protein	Dog	Eel	Dogfish	Brine shrimp
α Subunit (Mr)	97,000	97,700	104,200	97,800
β Subunit (Mr)	40,200	42,000	35,800	40,100
Holoenzyme (α_1 β_2)	274,400	279,400	280,000	275,800

a, Data from Peterson, G.L. and Hokin, L.E. (J.Biol. Chem. 256, 3751-3761, 1981)

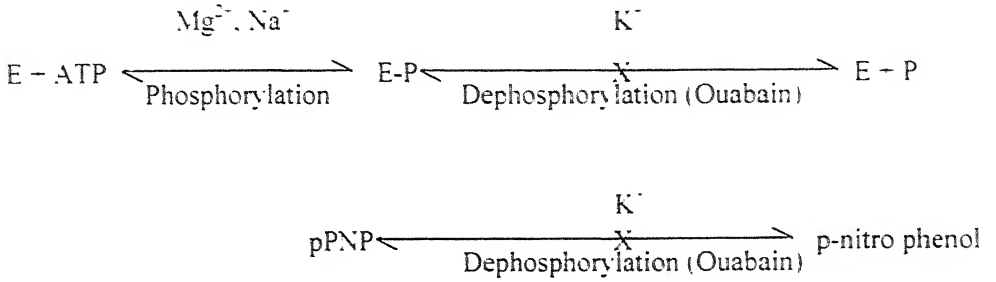
Na^+, K^+-ATPase contaqins 11-14 sulfhydryly groups/mole of αβ dimmer, of which 8-9 SH groups reside on the α subunit and 1-2 on the β subunit[54,59-61]. Using radioactive N-ethylmaleimide, it was determined that two to six sulfhydryl groups reside in the active center[54,61,62]. Alkylation of SH groups lead to the inactivation of Na^+, K^+-ATPase and its partial reactions, Na^+-dependent phosphorylation of the α subunit and K^+-activated phosphatase reaction[61-64,6,66]. So modification of free sulfhydryl groups in the α subunit of Na^+, K^+-ATPase has been found to inactivate the enzyme[67]

It is believed that β subunit may play an important role in the biosynthesis or assembly of functional pump molecule[68,69]. Trypsinolysis of the native Na^+, K^+-ATPase in absence of calcium and in the presence of rubidium ion, leading to the formation of a stable 19 kDa fragment, smaller embedded fragments of the α chain and an essentially intact β chain[70]. Stability of the β subunit in the native membrane to proteases (resistance to proteolysis) is mainly determined by existence of disulphide bonds[71]. Reduction of S-S bridges in the β subunit of the Na^+ pump results in a loss of occlusion and inactivation of the Na^+, K^+-ATPase[72,73]. So the extracellular domain of the β submit and its organization via S-S bridges is involved in an essential way in stabilizing the cation-occluded state of the Na^+ pump.

A large body of work over the last decades has attempted to establish the quaternary structure of the active enzyme unit [74-83]. Despite this intense focus, the question of whether the membrane-embedded Na^+, K^+-ATPase functions as an αβ protomer, an $(\alpha\beta)_2$ diprotomer of interacting α subunits or a higher order oligomers remain unresolved. Early work implicates the protomeric αβ-unit as the minimum asymmetric functional unit of the enzyme [74-76]. In contrast, molecular weight determinations using analytical ultracentrifugation[77], low angle laser light scattering[78] or radiation inactivation[79] and evidence from cross linking[80], ligand binding[81] and molecular distance measurements[82] support oligomeric model of Na^+, K^+-ATPase with an $(\alpha\beta)_2$ diprotomeric structure. However, in many cases, the existence of this oligomeric complexes could be due to nonspecific collisions of the αβ protomers in the densely packed membrane preparation and may not reflect specific functional subunit interactions. Recently, Koster *et al* has reported a cytoplastmic region of the Na^+, K^+-ATPase α subunit necessary for specific α/α association using the baculovirus expression system[83].

The survey of inhibitors of Na^+, K^+-ATPase system is helpful in the elucidation of the reaction mechanism. Digitalis glycoside, ouabain, displays a nearly absolute specificity for Na^+, K^+-ATPase and is used to determine the presence and function of this ATPase in a variety of tissues[22]. It does not inhibit other ATPase. It inhibits

binding of ATP[84] and dephosphorylation of the phosphointermediate[85] by reaction at the outer membrane side[86,87]. It inhibits p-nitrophenyl phosphatase activity. The activity is dependent on potassium and is comparable to the dephosphorylation step of Na^+, K^+-ATPase reaction (Scheme 2).



Scheme 2. Phosphorylation and dephosphorylation of Na^+, K^+-ATPase and the site of action of ouabain on the overall reaction scheme. A comparison is made with p-nitro phenyl phosphatase (p-NPPase) reaction.

In addition, sulphydryl reagents, oligomycin, butanedione and vanadate are known inhibitors of Na^+, K^+-ATPase[4]. Vanadate binds the ATPase from inside of the membrane. There are reports about ouabain-like inhibitor namely erythrophleum alkaloid commonly called cassaine. They are functionally similar but structurally different family of compounds[7]. Tetraethyl ammonium ion inhibits Na^+, K^+-ATPase from the outside in a voltage-dependent manner[88]. The enzyme has been found to be inhibited by different drugs[89-93] and hormones[94-96].

It is widely accepted that lipids perform an essential role in the functioning of the Na^+, K^+-ATPase[97], since it is a membrane bound enzyme. Removal of lipid from the membrane matrix by detergent, organic solvent or phospholipase treatment leads to partial or complete inactivation of the enzyme. The requirement of specific phospholipid(s) for activity[98] has been studied by delipidation of the crude enzyme, followed by reconstitution with various known phospholipids. It has been reported that phosphatidylserine is absolutely required for Na^+, K^+-ATPase activity in erythrocyte ghosts[99,100] but not in brain[101]. Phosphatidylinositol has been found to be involved for this enzyme functioning[102]. Mandersloot *et al*[103] identified phosphatidylinositol as the endogenous activator of Na^+, K^+-ATPase in rabbit kidney microsomes.

Fluidity of the membranes is found to be one of the important factors for regulating Na^+, K^+-ATPase activity [104,105], the β-fatty acid group influences the extent of enzyme reactivation[106]. Though cholesterol and other neutral lipids do not seem to be essential for the enzyme activity in a highly purified enzyme preparation, however, cholesterol has been found to influence the fluidity of the membrane and in some cases affect the activity.

Ca^{2+}, Mg^{2+}-and Ca^{2+}-ATPase

Calcium and magnesium activated ATPase (ATP phosphohydrolase, E.C. 3.6.1.3) which plays a role in Ca^{2+} ion transport, was discovered in 1961 by Hasselbach and Makinose[107]. The enzyme displays all the activites described for Na^+, K^+-ATPase an overall ATPase activity, pNPase activities, ADP-ATP exchange and phosphorylation by either ATP or Pi. All these activities depend on Ca^{2+} ion, except the phosphorylation by Pi, which is actually inhibited by Ca^{2+} ion[108].

The transport of calcium ion across cell membranes is shown if Fig. 3.

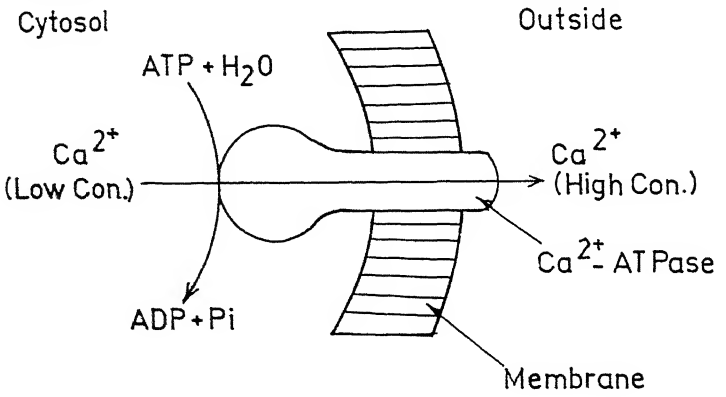


Fig. 3– Schematic diagram showing the transport of calcium ion across cell membranes. Con. Denotes concentration.

Ionized calcium in contrast to Na^+ and K^+ ions, affects a great variety of cellular functions at extremely low concentration. In erythrocytes and giant nerve and muscle fibers, the concentration of ionized Ca^{2+} was found to be lower than 1 μM. This concentration is more than 1,000 fold lower than that exists in the extracellular fluid. The very high concentration (millimolar range) of free Ca^{2+} ion in the extracellular pool as compared to that of intracellular milieu (submicromolar range) and the resulting very large electrochemical force on Ca^{2+} ion, are particularly convenient to its role as an intracellular regulator[109]. In sarcoplasmic reticulum, the enzyme

transports 2 Ca^{2+} ions at the expense of 1 mole of ATP[111]. For erythrocytes, efficiencies of 1 Ca^{2+} ion / ATP[112] and 2 Ca^{2+} ion / ATP[113] have been reported. At least seven Ca^{2+} ion transporting systems have been recognized in normal eukaryotic cells[113].

The reaction scheme of Ca^{2+}, Mg^{2+}, -ATPase is similar to that of Na^+, K^+ -ATPase involving binding of the substrate (step 1), phosphorylation (step 2), a conformational change with Ca^{2+} binding sites turning to the opposite side of the membrane (step 3) and hydrolysis of phosphoenzyme (step 4). In a nutshell, the reaction scheme can be shown as follows (Scheme 3)[111, 114-115].

$$E + APT \underset{[1]}{\overset{2Ca^{2+}(in)}{\rightleftharpoons}} E.MgATP.Ca_2 \underset{[2]}{\overset{ADP}{\rightleftharpoons}} E_1 \sim P.Mg.Ca_2$$

$$\underset{[3]}{\overset{Mg^{2+} + 2Ca^{2+}}{\rightleftharpoons}} \qquad E_2 - P \underset{[4]}{\overset{Pi}{\rightleftharpoons}} E$$

Scheme 3

The Ca^{2+}-ATPase in different organs are generally found to require Mg^{2+} ion for its activation[116,117]. The ATPase stimulated by external Ca^{2+} ion only without any Mg^{2+} ion, i.e. Mg^{2+} -independent Ca^{2+} -ATPase as it is commonly called has also been reported[118-121]. However, the exact role of this Ca^{2+}-ATPase in cellular functions is not known as of yet, however it has been reported recently from our laboratory that phosphorylation and dephosphorylation of the overall raction scheme is comparable to Ca^{2+}, Mg^{2+} -ATPase, however is controlled by low and high concentration of calcium instead of Mg^{2+} and Ca^{2+} (Scheme 4)[122].

$$E \overset{Ca^{2+}(low, in) + ATP}{\rightleftharpoons} E.Ca.ATP \overset{Ca^{2+}, ADP}{\rightleftharpoons} E_1 \sim P \overset{Ca^{2+}(high, out)}{\rightleftharpoons}$$

Scheme 4

Endogenous modulator proteins / peptides of ATPases

Several peptidic and nonpeptidic factors can regulate Na^+, K^+-ATPase activity[123-127]. However, ouabain, a cardiace glycoside is the most potent one[100]. In a very few cases

only, activation of Na^+, K^+ -ATPase by endogenous factors has been recorded. PEC-60, a novel regulatory peptide, isolated from procine intestine[128] can stimulate the Na^+, K^+ -ATPase activity from rat frontal cortex. Thus PEC-60 seems to be one of the endogenous activators that regulated this enzyme. Insulin[129-131], thyroxine[132], glucagon[131], bradykinin[133] and the N-terminal fragment of substance P (SPI-5)[134] have been found to activate Na^+, K^+ -ATPase most likely via receptor-mediated mechanism.

The regulator of Na^+, K^+ -ATPase can be divided into two groups :

(i) direct modulators of activity (ouabain, some proteins and peptides) and (ii) indirect modulators which include many peptides, nonpeptidic hormones and neurotransmitters etc[106,123]. The former group can bind directly with enzyme protein but the latter group of compounds affect via binding to membranes or specific receptors.

Na^+, K^+ -ATPase regulates several essential cellular functions such as intracellular homeostasis of Na^+ and K^+ ions, pH, membrane potential, cell volume, cellular uptake of amino acids and sugars in different tissues. Physiologically, in most cases, ATPase are controlled by endogenous regulator proteins[135]. Thus, an endogenous inhibitor protein inhibits porcine sperm motility[136] has been found to be a competitive inhibitor of Na^+, K^+ -ATPase and is identical to β-microseminoprotein. The enzyme is also inhibited by some endogenous glycosides[137,138] and some endogenous peptides of varying molecular masses[137]. A small peptide of molecular weight approximately 600 dalton isolated from human cerebrospinal fluid has been found to specifically inhibit Na^+, K^+-pump[137]. Arnaiz *et al*[139] reported that brain extract form rat either stimulates or inhibits Na^+, K^+ -ATPase. Our laboratory has isolated and characterized a number of low molecular mass (12-13kDa) potent inhibitor proteins of Na^+, K^+ -ATPase[140] and modulator protein of Ca^{2+}-ATPases[141] from rat brain. In addition, high molecular mass Na^+, K^+ -ATPase inhibitor proteins have been reported from our laboratory very recently[142,143].

It has been reprted by Cramber *et at*[144] that endogenous digitalis like factors (EDLF) specifically inhibit the Na^+, K^+ -ATPase activity. It differs from ouabain by three criteria : a preincubation with the membranes is required for full activity, no effect on the rat cerebral alpha 3 isoform and a steep dose response curve with the same apparent potency for rat alpha 2 and alpha 1 isoforms of high (10^{-7}M) and low affinity (10^{-5}M) for ouabain.

A compound of molecular mass of around 412.277 (as evident from mass spectrometric analysis) isolated from bovine hypothalamic and pituitary tissue and

structurally different from ouabain has been found to inhibit sodium pump[145]. The inhibitor besides inhibiting the sodium pump was found to play a role in the control of Calcium-homeostasis by direct modulation of transport systems implicated in the control of intracellular calcium. Unsaturated fatty acids such as oleic and palmitoleic acids are effective inhibitors of Na^+, K^+ -ATPase [146]

Scavone *et al* has reported that atrial natriuretic peptide (ANP) inhibits both active and passive movement of sodim ions through tubular lumen by sodium and potassium activated adenosine triphosphatase (Na^+, K^+ -ATPase)[147]. Modulation of Na^+, K^+ -ATPase by ANP is mimicked by 8-bromo cGMP and okadaic acid (OA) and is blocked by KT5823, a selective inhibitor of cGMP-dependent protein kinase (PKG), but not by KT5720, a selective inhibitor of cAMP dependent protein kinase (PKA) which suggests that the action of ANP on the sodium pump involves cGMP mediated changes in protein phosphorylation.

Several peptidic and non-peptidic factors are known to regulate Na^+, K^+ -ATPase. A number of them eg. Kaliuretic peptides[148] SPAI –1,2,3[149-151]. SMI-1, sperm motility factor[152] have been characterized. Brain extract[140] has been reported either to inhibit or stimulate Na^+, K^+ -ATPase activity. Recently an endogenous stimulator of Na^+, K^+ -ATPase, PEC-60 has been reported[128]. Non-peptidic regulators eg. Catecholamine[25, 153,154] vanadium[155], endogenous ouabain like glycosides[128 134, 132, 140, 152, 156, 157] have been reported to be very effective in inhibiting Na^+, K^+ -ATPase.

The other ion transporting ATPases e.g., Ca^{2+}, Mg^{2+} -and Ca^{2+} -ATPases have also been reported to be regulated by endogenous modulators. Narayanan *et al*[158] reported an endogenous protein inhibitor of sarcoplasmic reticulum calcium pump in heart muscle. A 12 kDa calcium binding protein from smooth muscle[159] and a membrane-intrinsic protein (phospholamban) from sarcoplasmic reticulum[160] have been reported as regulators of Ca^{2+} -ATPase. A 5 kDa peptide from *pendinus* was found to affect calcium-release channels of sarcoplasmic reticulum has been reported[161]. It has been demonstrated from our laboratory that a low molecular mass protein isolated from rat brain stimulates Ca^{2+}, Mg^{2+} -ATPase but inhibits Ca^{2+} -ATPase. When pooled fraction from sephadex G-100 containing a number of low molecular weight proteins were loaded on to Mono Q column in FPLC, a number of fractions were identified, collected from 0.1M hold NaCl eluent. One of the fractions of molecular mass of about 12 kDa was found to specifically stimulates Ca^{2+}, Mg^{2+} -ATPase and inhibits Ca^{2+} -ATPase activities with an S_{50} (concentration producing 50% stimulation) of about 0.65 nM and an I_{50} (concentration causing 50% inhibition) of about 0.35 mM respectively[141]. The modulator is found to bind to a site different from other calcium pump blockers like verapamil and trifluoroperazine. With respect to Ca^{2+}, Mg^{2+} -

ATPase, stimulatory effect was found to be reduced in presence of the above blockers, whereas, the inhibitory effect on Ca^{2+} -ATPase was found to be enhanced in the presence of these drugs.

The modulator was found to be stable upto 60 °C above which starts loosing its activity and binding to the ATPases are found to be reversible in nature. The modulatory effect of the regulator on Ca^{2+}, Mg^{2+} and Ca^{2+}-ATPases are due to the binding of the former to the latter and subsequent changes of conformation as was shown from fluorescence and CD-analysis[141]. So the modulator is found to be a good tool in differentiating the activities of Ca^{2+}, Mg^{2+} -ATPase and Ca^{2+} -ATPase.

Recent work from our laboratory suggests that endogenous proteins of different molecular masses isolated from different sources can either inhibit or stimulate ATPase activities.[140,41].

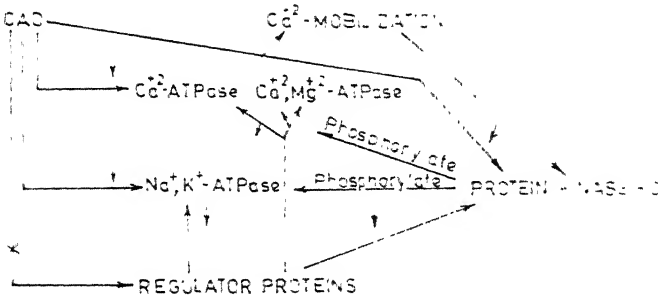
Thus a protein of molecular mass 12 kDa isolated and characterized from rat brain cytosol was found to inhibit Na^+, K^+-ATPase activity from diverse sources. The inhibitor does not have any effect on any other ATPases[140]. The inhibition was found to be due to change in conformation as evident from tryptophan fluorescence change which led to change in conformation of the enzyme molecule. Similarly, a protein of molecular weight about 13 kDa isolated from same source was found to stimulate Ca^{2+}, Mg^{2+} -ATPase activity while inhibiting Ca^{2+} -ATPase though both of them belong to SERCA family. The findings are extremely important in distinguishing the properties between Ca^{2+}, Mg^{2+} -ATPase and Ca^{2+} -ATPase[141]. The differential effect was found to be due to different types of binding leading to different conformational changes[141].

Very recently our laboratory has reported a protein of molecular weight 70-75 kDa isolated either from rat brain[143] or goat testis cytosolic fraction[175] are found to inhibit Na^+, K^+ -ATPase activity also. The inhibitor was found to inhibit the phosphorylation step of the ATPase reaction sequence. Furthermore our laboratory has done exhaustive study showing the inhibition of ion transporting ATPase by two amphiphilic drugs like chloroquine and chlorpromazine both *in vitro* and *in vivo* in different organs of rats[162-166]. As described above, the endogenous regulators modulated various ion transporting enzymes. The obvious question that comes in mind is whether during drug treatment, the inhibition of the ATPases are caused directly by these drugs or through the modulators. We have found that drug treatments (*in vivo* studies) do not affect the modulatory effect of the modulators at different time. It is therefore presumed that the inhibition of the ATPases by these drugs are

due to direct binding of the drugs with the ATPases which in turn inhibit the activites, however the effect was found to be reversible [5, 6].

Since the ATPases are found to be activated due to phosphorylation by protein kinases particularly by PKC[3,7-14], we explored the possibility of the effect of drugs on protein kinase. It has been found that PKC activity in rat brain is inhibited by about 25–30% after 5-6 weeks of these drug treatment. Therefore part of the inhibition of the ATPase activities by these drug treatment could be due to the defective phosphorylation of the ATPases by PKC and rest by direct drug binding to the enzymes as described above.

Based on the above findings, a scheme is proposed relating the mechanism of regulation of ion transporting ATPase by these drugs, endogenous modulators and protein kinase C (Scheme 5).



Scheme 5. Showing the regulation of ion transporting enzymes by cationic amphi-philic drugs (chloroquine and chlorpromazine), regulator proteins and protein kinase C.

CAD, Cationic amphiphilic drugs; ↑, stimulation; ↓, inhibition

References

1. Skou, J. C. (1957) *Biochim. Biophys. Acta* **23** : 394.
2. Green, N. M. (1992) *in Ion-Motive ATPase : Structure, Function and Rregulation*, eds. Scarpa, A., Carafoli, E. & Papa, S., New York Academy of Sciences, New York, p. 104.
3. Lane, L.K., Copehhaven, J. N., Lindenmayer, G.E. & Schwartz, A. (1973) *J. Biol Chem.* **248** : 7197.
4. Schwartz, A., Lindenmayer, G.G. & Allen J. C. (1975) *Pharmacol Rev.* **27** : 3.

5 Albers, R.W. (1967) *Annu. Rev Biochme.* **36** : 727.

6 Post, R. L., Kume, S., Tobin, T., Orcutt, B. & Sen, A. K. (1969) *J. Gen. Physiol.* **54** : 306s.

7 Post, R. L. (1981) in *Molecular Basis of Drug Action* (eds. Singer & Ondarza) Elsevier North Holland Inc., New York, p. 299.

8 Hegyvory, C. & Post, R. L. (1971) *J. Biol. Chem.*. **246** : 5234.

9 De Pont J. J. H.H.M., Schoot, B.M., Vanprooyen-Vaneeden, A. & Bonting, S.L. (1977) *Biochim Biophys Acta* **482** : 213.

10 Masiak, S.L. & Dangelo, G. (1975) *Biochim. Biophys Acta* **382** : 83

11 Patzeff-Wenczler, R., Pauls, H., Erdmann, E. & Schoner, W. (1975) *Eur J Biochem.* **53** : 301.

12 Cantley, L C. Jr., Gelles, J. & Josephson, L. (1978) *Biochemistry* **17** : 418.

13. Fahn, S., Koval, G. J. & Albers, R. W. (1966) *J. Biol. Chem.* **241** : 1882.

14. Mardh, S. & Zetterqvist, O. (1974) *Biochim. Biophys Acta* **350** : 473.

15. Tobin, T., Akera, T. & Brody, T.M. (1975) *Biochim. Biophyss Acta* **389** : 117.

16. Vasilets, L. A. & Schwartz, W. (1993) *Biochim Biophys Acta* **1154** : 201.

17. Siegel, G.J. & Albers, R. W. (1967) *J. Biol Chem.* **242** : 4972

18 Sen A. K. & Post, R. L. (1964) *J. Biol. Chem.* **239** : 345.

19. Jorgensen, P.L. (1974) *Biochim. Biophys. Acta* **356** : 53.

20 Kyte, J. (1971) *J. Biol. Chem.* **246** : 4157.

21 Lane, L. K. Anner, B.M., Wallick, E.T., Ray, M.V. & Schwartz, A. (1978) *Biochem. Pharmacol.* **27** : 225.

22. Bonting, S. L. (1970) in *Membranes and Ion Transport* (ed Bittar, E. E.) Willey Interscience, London. Vol. 1.p. 257.

23 Perrone, J. R., Hackney, J.F., Dixon, J.F. & Hokin, L. E. (1975) *J. Biol. Chem.* **250** : 4178.

24 Hopkings, B. E., Wagner, J. Jr. & Smith, J. W. (1976) *J Biol Chem.* **251** : 4365

25 Jorgensen, P. L. (1982) *Biochim. Biophys Acta* **694** : 26.

26. Marshall, P. J. & Hokin, L. E. (1979) *Biochim. Biophys. Res. Commun.* **87** : 476.

27. Dzhandzhugazyan, K.N., Modyanov, N. N. & Ovchinnikov, Yu. A. (1981) *Bioorg. Khim.* **7** : 847.

28. Linger, J. B., Orlowski, M., Shull, M.M., & Price, E.M. (1990) *Prog. Nucleic Acid Res. Mol. Biol.* **38** : 37.

29. Sweadner, K.J (1989) *Biochim. Biophys. Acta* **988** : 185.

30 Good, P. J., Richter, K. & Dawid, I.B. (1990) *Proc. Natl. Acad. Sci. U.S.A* **87** : 9088.

31 Jorgensen, P.L. & Andersen, J.P. (1988) *J. Membr. Biol.* **103** : 95.

32 Skou J.C. & Esmann, M. (1992) *J. Bioenerg. Biomembr.* **24** : 249.

33. Shull, G.E., Schwartz, A. & Lingrel, J.B. (1985) *Nature* **316** : 691.

34. Post, R. L. & Kume, S. (1973) *J. Biol. Chem.* **248** : 6993
35. Price E.M. & Lingrel, J.B. (1988) *Biochemistry* **27** : 8400
36. Price E.M., Rice, D.A. & Lingrel, J.B. (1990) *J. Biol. Chem.* **265** : 6638.
37. Zhang, Z., Devarajan, P., Dorfman, A.L. & Morrow, J.S. (1998) *J. Biol. Chem.* **273** : 18681
38. Devarajan, P., Stabach, P.R., De Matteis, M.A. & Morrow, J. S. (1997) *Proc. Natl. Acad. Sci. USA* **94** : 10711
39. Geering, K. (1990) *J. Membr. Biol.* **115** : 109
40. McDonough, A.A., Geering, K. & Farley, R.A. (1990) *FASEB J.* **4** : 1598
41. Geering, K. (1991) *FEBS Lett.* **285** : 189.
42. Lutsenko, S. & Kaplan, J.H. (1993) *Biochemistry* **32** : 6737
43. Eakle, K.A., Kabalin, M.A., Wang, S.G. & Farley, R.A. (1994) *J. Biol. Chem.* **269** : 6550.
44. Hasler, U., Wang, X., Crambert, G., Beguin, P., Jaisser, F., Horisberger, J.D. & Geering, K. (1998) *J. Biol. Chem.* **273** : 30826
45. Mercer, R.W., Biemesderfer, D., Bliss, D.P. Collins, H. & Forbush III, B. (1993) *J. Cell. Biol.* **121** : 579.
46. Therien, A.G., Goldshleger, R., Karlish, S.J.D. & Blostein, R. (1997) *J. Biol. Chem.* **272** : 32628.
47. Beguin, P. Hasler, U., Beggah, A., Horisberger, J.D. & Geering, K. (1998) *J. Biol. Chem.* **273** : 24921.
48. Horowitz, B., Eakle, K.A., Scheiner-Bobis, G., Randolph, G.R., Chen, C.Y., Hitzeman, R.A. & Farley, R.A. (1990) *J. Biol. Chem.* **265** : 4189.
49. DeTomaso, A.W., Xie, Z.J., Lui, G. & Mercer, R.W. (1993) *J. Biol. Chem.* **168** : 1470
50. DeTomaso, A.W., Balanco, G. & Mercer, R.W. (1994) *J. Cell Biol.* **127** : 55.
51. Lemas, M.V., Harmrick, M., Takeyasu, K. & Fambrough, D.M. (1994) *J. Biol. Chem.* **269** : 8255.
52. Wang, S.G. & Farley, R.A. (1998) *J. Biol. Chem.* **273** : 29400.
53. Craig, W.S. & Kyte, J. (1990) *J. Biol. Chem.* **255** : 6262.
54. Peter, W.H.M., Depont, J.J.H.M., Koppers, A. & Bonting, S.L. (1981) *Biochim. Biophys. Acta* **641** : 55
55. Peterson, G. & Hokin, L.E. (1981) *J. Biol. Chem.* **256** : 3751
56. Hayashi, Y., Takagi, T., Maezawa, S. & Matsui, H. (1983) *Biochim. Biophys. Acta* **748** : 153.
57. Cantley, L.C. (1981) *Curr. Top. Bioenerg.* **11** : 201
58. Freytag, J.W. & Reynolds, J.A. (1981) *Biochemistry* **20** : 7211.
59. Askari, A., Huang, W. & Henderson, G.R. (1979) in *Na, K-ATPase Structure and Kinetics* eds Skou, J.C. & Norby, J.G., Academic Press, London, p. 205
60. Kawamura, M., Ohta, T. & Nagand, K. (1983) *Curr. Top. Membr. Transp.* **19** : 153.

61 Jesaitis, J A & Fortes, P A.G. (1990) *J Biol Chem* **255** : 459.

62 Hart, W M. & Titus, E.O. (1973) *J Biol. Chem* **248** : 4674.

63 Skou, J.C & Hilberg, C. (1965) *Biochim Biophys Acta* **110** : 359.

64 Fahn, S , Koval, G.J & Albers, R.W. (1968) *J. Biol Chem* **243** : 1993

65 Banerjee, S P., Wong, S.M.E. & Sen, A.K. (1972) *Mol Pharmacol* **8** : 8.

66 Patzelt-Wenezler, R., Pauls, H , Erdmann, E &. Schoner, W. (1975) *Eur J. Biochem* **53** : 301.

67. Winslow, J W. (1981) *J Biol Chem.* **256** : 9522.

68 Geering, K , Meyer, D. I., Paccolat, M P., Krachenbuhl, J P & Rossier, B.C. (1985) *J Biol Chem* **260** : 5154.

69. Noguchi, S., Mishina, M., Kawamura, M. & Numa, S (1987) *FFBS Lett* **225** : 27.

70. Capasso, J.M., Hoving, S., Tal, D.M., Goldschleger, R. & Karlish, S J.D. (1992) *J Biol Chem* **267** : 1150

71. Arzamazova, N.M., Arystarkhova, E.A., Genondvan, N.M., Gavrilyeva, E. E. Azyzova, G. I., Cherlova, E.N., Klimenko & Modyanov, N.N. (1987) *Biol. Memb.* **4** : 590.

72. Kawamura, M. & Nagano, K. (1984) *Biochim Biophys. Acta* **774** : 188.

73. Kirley, T.L. (1990) *J. Biol. Chem.* **265** : 4227.

74. Martin, D.W. & Sachs, J. R. (1992) *J. Biol Chem* **267** : 23922.

75 Sachs, J R (1994) *Biochim Biophys Acta* **1193** : 199.

76 Vilsen, B , Andersen, J.P., Petersen, J & Jorgensen, P L (1987) *J Biol Chem* **262** : 10511

77. Esmann, M., Christiansen, C., Karisson, K. A., Hansoon, G. C. & Skou, J. C. (1980) *Biochim Biophys Acta* **603** : 1.

78. Hayashi, Y., Mimura, K., Matsui, H. & Takagi, T. (1989) *Biochim Biophys Acta* **983** : **217.**

79. Norby, J.G. & Jensen, J. (1991) in *The Sodium Pump : Recent Development* eds. Deweer, P. and Kaplan, J.H., Rockefeller university Press, New York, pp. 173

80 Huang, W. H. & Askari, A (1981) *Biochim Biophys Acta* **645** : 54.

81. Schoner, W., Thonges, D., Hamer, E., Antolovic, R., Buxbaum, E., Willeke, M., Serpersu, E. H & Scheiner-Bobis, G. (1994) in *The Sodium Pump : Structure, Mechanism, Hormonal Control and itsRole in the Disease,* eds Bamberg, E. and Schoner, W., , Springer, New York, pp. 332

82. Linnertz, H., Urbanova, P., Obsil, T., Herman, P., Amler, E. & Schoner, W. (1998) *J. Biol Chem.* **273** : 28813.

83 Koster, J C, Blanco, G. & Mercer, R.W. (1995) *J. Biol Chem.* **270** : 14332.

84. Hansen, O , Jensen, J. & Norby, J. G. (1971) *Nature. London, New Biol.* **243** : 122.

85 Sen, A K., Tobin, T. & Post, R. L. (1969) *J. Biol Chem.* **244** : 6596.

86. Caldwell, P. C. & Keynes, R. D. (1959) *J. Physiol. (London)* **148** : 8p.

87. Hoffman, J.F. (1996) *Am. J. Med* **41** : 666.

88. Eckstein-Ludwig, U., Rettinger, J., Vasilets, L. A., & Schwarz, W. (1998) *Biochim Biophys. Acta* **1372** : 289.

89. Giraud, F., Claret, M., Bruckdorfer, K. R. & Chailley, B. (1981) *Biochim Biophys Acta* **647** : 249.

90. Huang, H. C. (1981) *Clin Exp. Pharmacol Physiol* **8** : 567.

91. Dunst, J., Lullman, H. & Morh, K. (1983) *Biochem. Pharmacol* **32** : 1595.

92. Nakamura, S. & Racker, E. (1984) *Biochemistry* **23** : 385.

93. Mazumder, B., Mukherjee, S., NagDas, S. K. & Sen, P. C. (1988) *Biochem Int* **16** : 35.

94. Svoboda, P. & Mosinger, B. (1981) *Biochem. Pharmacol* **30** : 433.

95. Resh, M.D. (1983) *Biochemistry* **22** : 2781.

96. Cohen, M. P., Klepsur, H. & Shapiro, E. (1986) *Biochim. Biophys. Acta* **856** : 182.

97. Schurmans-Stekhoven, F. & Bonting, S. L. (1981) *Physiol Rev.* **61** : 1.

98. DePont, J. J. H. M., Van Prooijen-Van Eeden, A. & Bonting, S. L.(1978) *Biochim Biophys. Acta* **508** : 464.

99. Roelofsen, B. & VanDeenen, L.L.M. (1973) *Eur. J. Biochem* **40** : 245.

100. Taniguchi, K. & Tonomura, Y. (1971) *J. Biochem. (Tokyo)* **69** : 543.

101. DePont, J.J.H.H.M., Van Prooijen-Van Eeden, A. & Bonting, S.L. (1973) *Biochim Biophys. Acta* **323** : 487.

102. Wheeler, K P. & Whittam, R. (1970) *J Physiol. (London)* **207** : 303.

103. Mandersloot, J.G., Roelofsen, B. & DeGier, J. (1978) *Biochim. Biophys Acta* **508** : 478.

104. Grisham, C.M. & Barnett, R.E. (1973) *Biochemistry* **12** : 2635.

105. Walker, J.A. & Wheeler, K.P. (1975) *Biochim Biophys Acta* **394** : 135.

106. DePont, J.J.H.H.M., Peters, W.H.M. & Bonting, S.L. (1983) in *Current Topics in Membrane Transport*, eds. Bronner, F. & Kleinzeller, A. , Academic Press. Vol **19**, p 163

107. Hasselbach, W. & Makinose, M. (1961) *Biochem J* **333** : 518

108. Kanazawa, T. (1975) *J Biol. Chem.* **250** : 113.

109. Carafoli, E. (1975) *Annu. Rev Biochem.* **56** : 395.

110. Hasselbach, W. (1974) in *The Enzymes*, ed. Boyer, P.D. Academic Press. Vol. **10**, p. 431.

111. Schatzmann, H.J. (1975) *Curr. Top Membr. Transp* **6** : 135.

112. Quist, E.E. & Roufogalis, B.D. (1975) *FEBS Lett* **50** : 135

113 Carafoli, E. (1988) in *Methods Enzymol*, eds Fleischer, S. and Fleischer, B. Academic Press. Vol. **157** : p. 1.

114. Ikemoto, N (1975) *J. Biol Chem.* **250** : 7219

115. Meissner, G (1973) *Biochim. Biophys Acta* **255** : 19.

116. Niggle, V., Rooner, P., Carafoli, E. & Penniston, J.T. (1979) *Arch. Biochem. Biophys* **198 :** 124.

117. Joseph, S.K. & Williamson, J.R. (1983) *J.Biol Chem.* **258 :** 10425.

118. Verma, A.K. & Penniston, J.T. (1981) *J.Biol Chem.* **256 :** 1269.

119. Ochs, D.L. & Reed, P.W. (1984) *J.Biol Chem.* **259 :** 102.

120. Nagdas, S.K., Mukherjee. S., Mazumder, B. & Sen, P.C. (1988) *Mol. Cell Biochem.* **79 :** 161.

121. Sikdar, R., Ganguly, U., Pal, P., Mazumder, B. & Sen, P.C. (1991) *Mol. Cell. Biochem.* **103 :** 121.

122. Sikdar, R., Roy, K., Mandal, A. K. & Sen, P.C. (1999) *J. Biosc.* **24 :** 137.

123. Heber, E. & Heupert, G. (1987) *Hypertension* **9 :** 315.

124. Hernandez, R.J. (1992) *Neurochem. Int.* **20 :** 1.

125. Maeda, S., Nakamae, J. & Inoki, R. (1988) *Life Sci.* **42 :** 461.

126. Raynor, R.L., Zheng, B. & Kuo, J.F. (1991) *J. Biol. Chem.* **266 :** 2753.

127. Zeidel, M.L., Brady, H.R. Kone, B.C., Gullans, S.R. & Brenner, B.B. (1989) *Am. J. Physiol.* **257 :** C1101.

128. Kairane, C., Zilmer, M., Mutt, V. & Sillard, R. (1994) *FEBS Lett.* **345 :** 1.

129. Bernstein, H.H., Poeggel, G., Dorn, A., Luppa, H. & Ziegler, M. (1981) *Experientia* **37 :** 434.

130. Clausen, T. & Flatman, J.A. (1987) *Am. J. Physiol.* **252 :** E492.

131. Fehlmann, M. & Freychet, P. (1981) *J. Biol Chem.* **256 :** 7449.

132. Elkouby, A., Ledig, M. & Mandel, P. (1982) *Neurochem. Res.* **7 :** 387.

133. Brazy, P.C., Trellis, D.R. & Klotman, P.E. (1985) *J. Clin Invest.* **76.** 1812.

134. Wojtkowiak, R., Turska, E., Lachowich, L. & Koziolkiewicz, W. (190) *Gen Pharmacol.* **21 :** 403.

135. Shou, J.C. (1990) *FEBS Lett.* **268 :** 314.

136. Chih-Fang, C., Shean-Tai, C., Hellen, J. & Wen-Chang, C. (1996) *Biochem. Biophys. Res. Commun.* **216 :** 623.

137. Goto, A., Yamada, K., Yagi, N., Yoshioka, M. & Sugimoto, T. (1992) *Pharmacol. Rev.* **44 :** 377.

138. Zhao, N., LO., L.C., Berova, N., Nakanishi, K., Tymiak, A.A., Ludens, J.H. & Haupert Jr. G.T. (1995) *Biochemistry* **34 :** 9893.

139. De Lores Arnaiz, G. R., De Lima, M. A. D. G. & Girardi, E. (1988) *Neurochem. Res.* **13 :** 229.

140. Bhattacharyya, D. & Sen, P.C. (1997) *Eur. J. Biochem.* **244 :** 829.

141. Bhattacharyya, D. & Sen, P.C. (1998) *Biochem. J.* **330 :** 95.

142. Chandra, S., Adhikary, G., Sikdar, R. & Sen, P.C. (1993) *Biochim. Biophys. Acta.* **1144 :** 33.

143. Roy, K., Mandal, A.K. & Sen, P.C. (1999) *Eur. J. Biochem.* **261 :** 84.

144. Cramber, G., Balzan, S., Paci, A., Decollogne, S., Montali, U., Ghione, S. & Lelievre, L.G. (1998) *Clin Expt Hypertens* **20** : 669.

145. Sancho, J.M (1998) *Clin Exp Hypertens*, **20** : 535.

146. Burth, P., Younes-Ibrahim, M., Goncale, F H., Costa, E.R. & Faris, M.V. (1997) *Infec. Immun.* **65** : 1557.

147 Scavone, C , Scanlon, C., McKee, M & Nathanson, J.A., (1995) *J. Pharmacol. Exp.* **272** : 1036

148. Shirley, C & Vesely, D.L. (1996) *Endocrionology* **136** : 2033.

149. Araki, K., Kuroki, J , Ito,O., Kuwada, M. & Tachibana, S. (1989) *Biochem. Biophys. Res. Commun* **164** : 496.

150. Araki, K., Kuwada, M., Ito,O., Kuroki, J. & Tachibana, S. (1990) *Biochem. Biophys. Res. Commun* **172** : 42

151 Ishizuka, N., Fukushima, Y., Urayama, O & Akera, T. (1991) *Biochim. Biophys. Acta* **1069** : 259.

152. Chih-Fang, C , Shen-Tai, C., Hellen, J. & Weng-Chang, C. (1996) *Biochem Biophys. Res. Commun* **218** : 623.

153. Cantley, jr. L.C., Furguson, J.H. & Kustin, K. (1978) *J Am Chem. Soc.* **100** : 5210.

154. Horwitz, B.A. (1979) *FASEB* **38** : 2170.

155. Cantley, jr. L.C., Cantley., L.G. & Josephson, L. (1978) *J. Biol. Chem* **253** : 7361.

156. Tal, D.M., Katchalsky, S., Lichtstein, D & Karlish, S.J.D. (1986) *Biochem Biophys Res. Commun* **135** : 1.

157. De Lores Arnaiz, G.R. , Lima, M.A.D.G. & Giradi, E. (1988) *Biochem. Res* **13** : 229.

158. Narayanan, N., Lee, P., Newland, M. & Khandelwal, R. (1982) *Biochem. Biophys Res. Commun.* **108** : 1158.

159. Mani, R.S & Kay, C.M. (1992) *Arch Biochem Biophys.* **296** : 442.

160 Szymanska, G., Kim, H.W., Cuppoletti, J. & Kranias, E G. (1992) *Mol. Cell Biochem* **114** : 65.

161. Valvia, H.H , Kirby, M.S., Laderer, W.J. & Coronado, R (1992) *Proc Natl Acad Soc USA* **89** : 12185.

162. Mazumder, B., Mukherjee, S & Sen, P.C. (1980) *Mol Cell Biochem* **95** : 13

163. Mazumder, B., Sikdar, R. & Sen, P C (1991) *Ind J Biochem Biophys* **28** : 136

164. Adhikary, G., Chandra, S., Sikdar, R., Nandy, P. & Sen, P C. (1991) *Biochem. Int* **25** : 951

165. Chandra, S., Adhikary, G. Sikdar, R. & Sen, (1992) *Mol Cell. Biochem* **118** : 15.

166 Adhikary, G., Chandra, S., Sikdar, R. & Sen, P.C. (1994) *Biochim Biophys Acta* **1188** : 220.

167. Pedemonte, C H , Pressley, T A. Lokhandwala, M.F. & Cinelli, A.R. (1997) *J Memb Biol* **155** : 219

168. Belusa, R , Wang, Z.M., Matsubara, T., Sahlgren, B., Dulubova, I., Nairn, A.C., Ruoslahti, E., Greengard, P. & Aperia, A. (1997) *J Biol Chem* **272** : 20179.

169 Chibalin, A.V., Pedemonte, C.H., Katz, A.I., Feraille, E., Berggren, P.O. & Bertorello A.M. (1998) *J Biol Chem.* **273 :** 8814.

170. Chibalin, A.V., Ogimoto, G., Pedemonte, C.H., Pressley, T.A. Katz, A.I., Feraille, E. Berggren, P O. & Bertorello, A.M. (1999) *J. Biol Chem.* **274 :** 1920

171 Owada, S., Larsson, O., Arkhammar, P. Katz, A.I., Chibalin, A.V., Berggren, P.O. & Bertorello, A M. (1999) *J. Biol. Chem.* **274 :** 2000.

172 Narayanan, N. & Xu, A. (1997) *Basic Res. Cardiol.* **1 :** 25.

173. Wang, K.K.W., Wright, L.C., Machan, C.L. Allen, B.G., Conigrave, A.D. & Roufogalis, B.D. (1991) *J Biol Chem.* **266 :** 9078.

174. Rogue, P.J. Humbert, J.P., Meyer, A., Freyermith, S., Krady, M.M. & Malviya, A.N. (1998) *Proc Natl. Acad. Sci. USA* **95 :** 9178.

175 Mandal, A K., Roy, K., Sir, P.C., Yadav, S. & Sen, P.C. (2001) *Mol. Cell. Biochem.* **223 :** 7.

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Hybridization studies between different cultivars and wild species of *Solanum* (egg plant)

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Abstract

Intra- and interspecific crosses were attempted between several varieties and or species of *Solanum* (egg plant). In intervarietal crosses (100-150 trials), the per cent successful crosses ranged from 62-87%, but only 40-70% fruits attained maturity. In attempts made between different *Solanum* cultivars and wild species, successful hybrid with seeds resulted from cross between *S melongena* cv. Pusa Purple Cluster and *S incanum* (wild species). Most of the intervarietal crosses showed higher crossability index. Marked increase in expression of heterosis was also encountered in hybrids resulting from parents widely different in morphological traits.

(**Keywords :** hybridization/*Solanum*/hybrids, crossability index, heterosis)

Introduction

Hybridization has played pivotal role in the improvement of crop plants. It plays a vital role in overcoming the food storage problem due to unabated population pressure of the country. It needs selection of compatible parents, which can be deciphered by floral characteristics and genome analysis. This allows selection of parent to permit transfer of desirable gene(s) from donor to the recipient. In recent years, the desirable characteristics of wild forms have drawn the attention of breeders to attempt hybridization with a view to exploiting such characteristics, but the success achieved so far in *Solanum melongena* has been non-existent or else only partial fertile hybrids could be obtained[1-4].

Several interspecific hybridization between different members of *Solanum* were attempted in India and abroad[5-13], but only a few crosses with fertile hybrids could be

realized due to crossability barriers. In addition, cross-incompatibility, sterility of the species, self-incompatibility, involvement of lethal genes and the other genetic factors or genomic distance are the other known barriers to species hybridization. Many primitive, but desirable characters involving disease resistance, productivity and ecological tolerance could possibly by introduced into cultivated lines through hybridization with wild germplasms of the egg plant as a number of wild taxa have been reported to possess fair affinity with the cultivated *S. melongena* varieties[14-16]. The success of crosses generating fertile hybrids is measured by the ability of parents to cross which is presented by crossability index[17]

The phenomenon of heterosis, commonly referred to as hybrid vigour is well known and has been extensively exploited by the breeder for higher production[18-20]. The analysis of combining ability helps the breeder in selecting suitable parents for hybridization and characterizing the nature and magnitude of gene action in the expression of traits. Several workers[21-22] have studied the general and specific combining abilities of brinjal with respect to various important parameters, like yield potential, flowering, fruit setting, plant height, etc.

In the present investigation, five wild and five cultivates of *Solanum* sp. (egg plant) were selected for intervarietal and interspecific crosses to study crossability index and magnitude of heterosis.

Materials and Methods

Based on morphological and yield and yield attributing characters five wild species viz., *S. incanum, S. indicum, S. sisymbrifolium, S. surattense* and *S. torvum* and five cultivars of *S. melongena* viz., Banaras Giant, Bangladesh Green Long, Pusa Kranti, Pusa Purple Cluster nad Pusa White Oval were chosen for hybridization experiments. For controlled intervarietal and interspecific crosses suitable flower buds were selected (a day prior to the opening of flowers) for emasculation purposes. Anthers were removed carefully without injury of female parts in the evening and the emasculated buds were kept enclosed in butter paper bags until pollination. Next day morning, pollens from freely open flowers of desired male parents were applied gently to the stigma of the emasculated long styled flowers and again bagged to avoid any foreign pollen grains. The pollinated flowers were tagged properly. This practice was performed for three consecutive days so as to ensure pollination. The bags were removed after 7-10 days. For successful crosses a crossability index used to measure the crossing affinity between each pair of parents was prepared. This index was derived from commonly used

seed set crossability index[17] i.e. % seed set in crosses / % seed set in selfs × 100. All parental species/ varieties and their hybrids were grown side by side in open field conditions. The data presented are based on observations made with ten random plants from each category and the values of particular parameters indicate means of ten readings per plant. Plant height and yield and its attributing characters viz., fruit length and width, single fruit weight, number of fruits plant^{-1}, number of seeds fruit^{-1} and the total yield plant^{-1} were considered and recorded. Data were analysed and mean comparison of each characteristic was made by Duncan's Multiple Range Test.

Estimation of Heterosis

The magnitude of heterosis (per cent) was measured in terms of heterosis over better parent [Het. (BP)] by using the following formula

$$\text{Het. (BP)} = \frac{F_1 - BP}{BP} \times 100$$

where,

F_1 = Mean performance of F_1

BP = Mean performance of better parent

Test of Significance

Significance of heterosis was tested by computing 't' as given below :

$$\text{'t' (BP)} = \frac{F_1 - BP}{\text{S.E. of heterosis over BP}}$$

The calculated 't' value was compared with the table value of 't' for significance test.

Results

Intra- and interspecific crosses were attempted between several varieties and/or species of *Solanum* viz., *S. melongena* (var. Banaras Giant, Pusa White Oval, Pusa Purple Cluster, Pusa Purple Long, Pusa Kranti and Bangladesh Green Long), *S. incanum*, *S. indicum*, *S. sisymbrifolium*, *S. torvum* and *S. surattense*. Of the intervarietal crosses (100-150) trials of *S. melongena* made between Pusa White Oval × Pusa Purple Cluster, Bangladesh Green Long × Pusa Kranti, Pusa Kranti × Banaras Giant and Pusa Purple Cluster × Pusa Kranti, the per cent successful crosses ranged from 62-87%, but only 40-70% fruits attained maturity, while the remaining fell down. Likewise, 80-100 attempts were made between *S. melongena* cultivars and wild species and among wild species, but successful hybrid seeds could be obtained from crosses between S. *incanum* and *S. melongena* cv. Pusa Purple Cluster only (Table 1).

Table 1 – Crossability relationships between different cultivars and wild species of *S. melongena*.

Crosses (Cultivar × Cultivar, Cultivar × Wild Species, Wild Species × Wild Species)	No. of attempted crosses	per cent successful crosses	Per cent fruits attained maturity	Crossability index
cv. Pusa White Oval × cv. Banaras Giant	145	81	70	67.82
cv. Bangladesh Green Long × cv. Pusa White Oval	124	73	61	63.21
cv. Pusa White Oval × cv. Pusa Purple Cluster	150	87	73	71.33
cv. Bangladesh Green Long × cv. Pusa Kranti	102	66	44	41.12
cv. Pusa Purple Cluster × cv. Pusa Kranti	115	71	56	62.55
cv. Pusa Kranti × Banaras Giant	100	62	40	31.03
S. incanum × cv, Pusa Purple Cluster	80	43	19	17.34

Most of the intervarietal crosses showed higher corssability index. The crossability index was found to be maximum (71.3) between c. Pusa White Oval × Pusa Purple Cluster, whereas the crossability index recorded for crosses between *S. incanum* × *S. melongena* cultivars was comparatively quite low (17.3).

The following intervarietal combinations produced hybrid seeds :

(a) cv. Pusa White Oval × cv. Banaras Giant
(b) cv. Bangladesh Green Long × Pusa White Oval
(c) cv. Pusa White Oval × cv. Pusa Purple Cluster
(d) cv. Bangladesh Green Long × cv. Pusa Kranti
(e) cv. Pusa Kranti × cv. Banaras Giant
(f) cv. Pusa Purple Cluster × cv. Pusa Kranti

Morphology of F_1 Hybrids

cv. Pusa White Oval × cv. Banaras Giant

The morphological features of hybrids resembled those of parents, except the shape and colour of fruit, which were oblong and light green, respectively. While most of the parameters under study showed intermediate characters, the plant height (88 cm.) was slightly higher than that of the parent cv. Banaras Giant (86 cm.) The number of seeds fruit^{-1} decreased, while the yield registered a significant increase (4161 g) in the hybrids.

cv. Bangladesh Green Long × cv. Pusa White Oval

The hybrid plants looked similar to their parents morphologically. Fruits were solitary and elongated in shape similar to female parent (cv. Bangladesh Green Long), but the colour of fruits was green and striped. Most of the parameters in hybrids were found to be insignificant compared to cv. Bangladesh Green Long, except single fruit weight. On the other hand, total yield plant^{-1} significantly decreased (1300 g) in the hybrid.

cv. Pusa White Oval × cv. Pusa Purple Cluster

The hybrids resembled the parents in several morphological characters. Hybrid plants were erect and partially spiny in habit with flowers and fruits borne in clusters similar to cv. Pusa Purple Cluster; the fruits, however, developed light green color. Heterotic effects were exhibited by the hybrid with regard to plant height (80 cm) and total productivity (4334 g) over parents. Likewise, average

number of fruits plant^{-1} and the length of fruits also showed some increase over better parents.

cv. Bangladesh Green Long × cv. Pusa Kranti

The hybrid plants morphologically resembled their parents as erect and spineless plants. Flowers are solitary in nature and purple in colour which develop into elongated fruits. The yield and yield attributing characters viz., fruit length, number of fruits plant^{-1} and total yield plant^{-1} reduced sharply, whereas the rest characters exhibited an intermediate range except plant height that measured smaller than that of either parents.

cv. Pusa Kranti × cv. Banaras Giant

The hybrid looked morphologically similar to the parents, but was shorter in height and showed extreme reduction in its characters. Marked reduction was observed in the yield and yield attributing characteristics, except the average number of fruit plant^{-1} which showed intermediate values.

cv. Pusa Purple Cluster × cv. Pusa Kranti

The gross morphological features of the hybrid were similar to their parental lines. Fruits occurred in clusters, like Pusa Purple Cluster and were round in shape. Most of the parameters considered were found to be intermediate with respect to their parents. Extreme reduction in total yield was observed in the hybrid compared to parents.

The values of best hetrotic crosses and the mean values of parents and hybrids are given in Table 2. The F_1 progenies usually exhibited negative heterobeltoisis effects over better parents for different parameters studied. The F_1 progenies of cv. Pusa White Oval × cv. Pusa Purple Cluster showed hetrobeltiosis for plat height (11%) which was significant at 5% level. Likewise, total yield of F_1 progenies of the crosses cv. Pusa White Oval × cv. Pusa Purple Cluster increased significantly compared to better parents (Table 2).

In F_2 generation, the plants exhibited wide range segregation, particularly for plant height and fruit characters, thus providing an opportunity for selection of plants with improved characters.

Table 2 – Heterotic effects over better parent for various characters viz., plant height, fruit length, fruit width, fruit weight, number of fruits plant^{-1} and total yield plant^{-1} in the crosses attempted.

Characters	Crosses	Parent mean	Hybrid mean	Heterotic effect over better parent	Heterobel-tiosis (%)
Plant height (cm)	PWO × BG	72.03 × 85.73	88.20	2.47^{NS}	2.88
	BGL × PWO	74.43 × 72.03	75.40	-2.03^{NS}	-2.62
	PWO × PPC	72.03 × 69.90	80.17	8.14*	11.30
	BGL × PK	77.43 × 77.07	75.25	-2.18^{NS}	-2.81
	PK × BG	77.07 × 85.73	73.07	-12.66**	-16.43
	PPC × PPC	69.90 × 77.07	72.34		-6.14
Fruit length (cm)	PWO × BG	8.55 × 13.13	11.87	-1.26^{NS}	-9.60
	BGL × PWO	25.25 × 8.55	22.78	-2.47^{NS}	-9.78
	PWO × PPC	8.55 × 8.80	10.55	1.75^{NS}	19.89
	BGL × PK	25.25 × 13.88	8.07	-17.18**	-68.03
	PK × BG	13.88 × 13.33	7.30	-5.81	-41.86
	PPC × PPC	8.80 × 13.88	9.21	-4.67**	-33.64
Fruit width (cm)	PWO × BG	4.69 × 11.07	9.78	-1.29^{NS}	-11.65
	BGL × PWO	2.86 × 4.69	2.31	-2.38**	-50.75
	PWO × PPC	4.69 × 2.99	3.58	-1.11*	-18.12
	BGL × PK	5.86 × 5.75	3.27	-2.48*	-43.13
	PK × BG	5.75 × 11.07	4.87	-6.20*	-56.00
	PPC × PPC	2.99 × 5.75	4.21	-1.54*	-26.78
Single fruit weight (g)	PWO × BG	108.22 × 475.49	372.49	-103.00***	-21.66
	BGL × PWO	133.87 × 108.22	110.66	-23.21**	-17.34
	PWO × PPC	108.22 × 44.60	77.17	-31.05**	-28.69

Table 2 Continued

	BGL × PK	133.87 × 125.85	82.75	-51.12**	-38.19
	PK × BG	125.85 × 475.49	80.70	-394.79**	-83.02
	PPC × PPC	44.60 × 125.85	69.25	-56.60**	-44.97
Number of fruit plant^{-1}	PWO × BG	31.84 × 6.09	10.21	-21.64**	-67.96
	BGL × PWO	15.33 × 31.84	13.11	-18.73**	-58.82
	PWO × PPC	31.84 × 48.89	53.21	+4.32^{NS}	+8.84
	BGL × PK	15.33 × 14.33	7.02	-8.31^{NS}	-54.20
	PK × BG	14.33 × 6.09	6.57	-7.78**	-45.85
	PPC × PPC	48.89 × 14.33	16.01	-32.88**	-32.75
Total yield plant^{-1}	PWO × BG	3245.72 × 2950.75	4161.46	-915.75*	28.21
	BGL × PWO	1900.22 × 3245.72	1300.36	-1945.36**	-59.94
	PWO × PPC	3245.72 × 2551.15	42.33.67	+987 95*	30.44
	BGL × PK	1900.22 × 1953.34	691.36	-1261.98**	-64.60
	PK × BG	1953.34 × 2950.75	569.19	-2381.56**	-80.71
	PPC × PPC	2551.25 × 1953.34	1289.34	-1261.91*	49.46

Note : Level of Significance : *-significant at p<0.005; **-significant at p<0.01; ***-significant at p<0.001; NS-non significant.

Discussion

In order to fight the menace of hunger and malnutrition, increase in yield and better nutritive value of produce are the important aspect of hybridization process. Stebbins[23] and Falusi and Morakinyo[24] reported interspecific hybridization as an important process for induction of variability, while, Kalloo *et al.*[25] and Singh *et al.*[26] described intervarietal hybridization as potent method of crop improvement both qualitatively and quantitatively.

Most of the crosses between *S. melongena* cultivars and wild allies and amongst wild relatives were found to be unsuccessful. The only exception was crosses between

S. melongena cultivars and *S. incanum*, which materialized, but with low crossability index. Intervarietal crosses, however, represented fairly high degree of crossability index in this study (Table 1). This indicates the existence of some barriers between the spinous and non-spinous species of *Solanums* even if the chromosome number is same. Failure of crosses can be ascertained on the basis of complete failure of fruit setting, parthenocarpic fruit setting, production of shrunken seeds, production of well developed, but non-germinating seeds and seedling mortality. Besides, cross-incompatibility, fertility of the species, self-incompatibility, involvement of lethal genes and some other genetic/genomic factors are barriers to species hybridization as reported earlier[6,27,28].

Anaso and Uzo[28] and Hasan and Lester[29] reported close similarities between *S. melongena* and *S. incanum*, both morphologically and genomically. Pearce and Lester[14] also established chemotaxonomic closeness between wild *S. incanum* and the cultivated *S. melongena* and considered the former species to be an intermediate between wild allies and cultivated *S. melongena*.

The results of these observations also confirm the present findings on crossability relationship between *S. melongena* and *S. incanum*. The incompatibility between *S. indicum* and *S. melongena* observed in this investigation was supported by several workers, but there exist several conflicting reports regarding the compatibility system between *S. melongena* and *S. indicum*. While Bhaduri[30] and Magoon *et al.*[31] reported incompatibility between the two species, Rangaswamy and Kadambavanasundaram[2] observed complete sterile to semi-fertile hybrids crossing the said species. In contrast, Rao and Kumar[32] reported fully fertile hybrids, based on crosses made by them.

Devis and Heywood[33] reported that crossability is influenced by minor genetic factors rather than by total genotypic constitutions. Crossability between the species, therefore, not necessarily indicates the cytogenetic relationship of the taxa or their systematic positions. Consequently, for successful crosses a measure of the affinity between the constituent parents, based on their ability to cross and produce hybrids, was devised and presented in the form of crossability index[17].

The success of most of the crosses attempted here between different cultivars and varieties of *S. melongena* indicated the cross compatible nature of the forms studied. The cross compatibility of the species and vigour of the hybrids with fertile pollens and fruit- and seed-set further indicated that the species and their allies under reference are closely related.

In the present study, the morphological as well as yield attributing characters of F_1 hybrids were found to usually represent intermediate character which direct their genomic relationships with their respective parents and proves the degree of genetic differences at varietal level. However, apparent variations occurred in the fruit colour and total yield.

It is noticed that in the hybrid (Pusa White Oval × Banaras Giant), most of characters showed insignificant difference with cv. Banaras Giant, indicating its close genetic similarity with one of its parents i.e. cv. Banaras Giant. Similarly, hybrids (Bangladesh Green Long × Pusa White Oval and Pusa White Oval × Pusa Purple Cluster) showed non-significant variations in their characters with the parent cv. Bangladesh Green Long and cv. Pusa Purple Cluster, respectively.

On the other hand, in the hybrids (Bangladesh Green Long × Pusa Kranti and Pusa Kranti × Banaras Giant), the intensity of parameters were significantly reduced compared to both the parents indicating less genetical similarity. However, in hybrid Pusa Purple Cluster × Pusa Kranti, 50% of the characters appeared from each parent; this suggests that the hybrids share genomic relations with both of the parents. These findings indicate that the genetic relationship of a variety may be expressed on the basis of per cent success of intervarietal crosses ad production of fertile hybrids. The crossability relationship at varietal level also indicates that the varieties evolved from the same genetic stock.

Heterosis is the superiority of F_1 hybrids over the parents involved and genetic systems of vegetable crops have definite advantages to exploit heterosis for desired characteristics. Negative heterosis is utilized for earliness in characteristics, like flowering or maturity of fruits, while positive heterosis is exploited for other commercially important attributes. Heterosis in eggplant was shown by several workers[18,34]. In this study, out of six hybrids produced only two (Pusa White Oval × Pusa Purple Cluster and Pusa White Oval × Banaras Giant) proved to be commercially beneficial and exhibited heterobeltiotic effects (improvement over better parent) for total productivity from multiplicative interaction of yield components i.e. number, size and weight of fruits. In the hybrid Pusa White Oval × Pusa Purple Cluster, plant height also showed heterobeltiotic effect (Table 2). There exists marked increase in the expression of heterosis when parents differ widely in morphological traits. It was noticed that high-yielding hybrids could be obtained by crossing high-yielding parents[35]. Hybrid vigour in plants is well known today and the breeders have been exploiting it since long for higher yield in several crops. Even to-day when several biotechnological approaches are known for bringing out spectacular improvements in

the quality and quantity of vaious commodities required for better and sustained human life, conventional hybridization procedure are still more popular and efficient method of crop improvement.

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References

1. Rajasekharan, S. (1971) *Euphytica* **19** : 217.
2. Rangaswamy, P. & Kadambavanasundaram, M. (1974) *Caryologia* **39** : 645.
3. Ramanna, M.S. & Hermsen, J.G. (1979) *Euphytica* **29** : 9.
4. Lopez, I. & Hawkes, J.G. (1991) *Solanaceae.* (The Royal Botanical Gardens, Kew, Richmond, Surrey U.K.) **111** : 327
5. Kirti, P.B. & Rao, B.G.S. (1980) *Caryologia* **33** : 289
6. Omidiji, M.O. (1975) *Euphytica* **24** : 341.
7. Omidiji M.O. (1983) *Cytologia* **48** : 35.
8. Ganapathi, A. & Rao, G.R. (1987) *Cytologia* **54** : 91.
9. Singh, R.N. & Roy, S.K. (1986) *Cytologia* **53** : 85.
10. Kumar, N. & Ram, H.H. (1987) *Ind J. Agric. Sci* **57** : 89.
11. Mathew, T.O. & Kuriachan, P. (1989) *Cytologia* **54** : 661.
12. Mausuelli, R.W. & Camadro, E.L. (1992) *Cytologia* **57** : 161.
13. Bukenya, Z.R. & Carasco, J.F. (1995) *Euphytica* **86** : 5.
14. Pearce, K.G. & Lester, R.N. (1979) in - "*The Biology and Taxonomy of the Solanaceae*", eds. Hawkes, J.G., Lester, R.N. & Skelding, A.D., Academic Press, London, p. 615.
15. Anis, M., Baksh, S. & Iqbal, M. (1994) *Cytologia* **59** : 433.
16. Rao, G.R. (1981) *Proc. Nat.Acad. Sci. Ind.* **56** : 141.
17. Marks, G.E. (1965) *New Phytol.* **64** : 293.
18. Singh, B. & Kumar, N. (1988) *Veg. Sci.* **15** : 72.
19. Mandal, A.K., Pandit, M.K. & Maity, T.K. (1994) *Crop Res. Hissar* **8** : 291.
20. Singh, D.P. & Prasad, V.S.R.K. (1995) *Ind. J. Hort.* **52** : 291.
21. Padamnabham, V. & Jagadish, C.A. (1996) *Ind. J. Genet.* **56** : 141.

22. Srivastava, J.P., Singh, H., Srivastava, B.P. & Verma, H.P.S. (1998) *Veg. Sci.* **25** : 45.
23. Stebbins, G.L. (1959) *Proc. Amer. Phil. Soc.* **103** : 231.
24. Falusi, O.A. & Morakinyo, J.A. (1994) *African Crop Sci. J.* **2** : 169.
25. Kalloo, G., Sharma, N.K. & Baswana, K.S. (1993) *Ind. Hort.* **38** : 10.
26. Singh, A., Singh, P.K., Dixit, J. & Gautam, J.P.S. (1995) *Hort. J.* **8** : 125.
27. Rao, S.V. & Rao, B.G.S. (1984) *Theo. Appl. Genet* **67** : 419.
28. Anaso, H.U. Ozo, J.O. (1990) *Cytologia* **55** : 1.
29. Hasan, S.M.Z. & Lester, R.N. (1992) *Acta Hort.* **292** : 123.
30. Bhaduri, P.N. (1951) *Ind. J. Genet. Pl. Breed.* **11** : 75.
31. Magoon, M.L., Ramanujam, S. & Cooper, D.C. (1962) *Caryologia* **15** : 151.
32. Rao, G.R. & Kumar, A. (1980) *Proc. Ind. Acad. Sci.* **89** : 117.
33. Devis, P.H. & Heywood, V.H. (1967) *Principles of Angiosperm Taxonomy*, Oliver and Boyd, Edinburg & London.
34. Chadha, M. L. & Hegde, R.K. (1989) *Ind. J. Hort.* **46** : 44.
35. Salehuzzaman, M. & Subash, K. (1982) *Philos. J. Biol.* **7** : 39.

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Comparative analysis on food, energy and nitrogen intake of a megachiropteran bat, *Cynopterus sphinx*

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Abstract

The short-nosed fruit bat, *Cynopterus sphinx* stated to feed about 60 min after being offered fruits in captivity. Food intake differed across four different varieties of fruits and ranged from 60% to 140% of the body mass. Bats ingested an average of 66.2 ± 21.8 g of *Carica papaya*, 59.3 ± 7.3 g of *Psidium guajava*, 25.8 ± 9.8 g of *Achras sapota* and 24.4. ± 8.2 g of *Musa paradisiaca* when these fruits were offered individually. In addition to the quantity of food intake, differences were observed in consumption of energy and carbohydrate but there was no difference in the uptake of nitrogen. Bats ingested high quantities of low-energy and nitrogen poor fruits such as *C papaya* and *P. guajava* compared to energy and nitrogen rich fruits such as *M. paradisiaca* and *A sapota*, presumably to balance their nitrogen requirement. Our results show that the quantity of food intake is influenced by the requirement of nitrogen.

(**Keywords** : fruit bats/foraging behaviour/food choice/energy and nitrogen requirement/ *C.sphinx*)

Introduction

The Old World fruit bat family Pteropodidae consists of approximately 150 species, most of which are obligate frugivores and nectarivores. However, nitrogen rich diets such as insects and liquid fractions of leaves have also been reported in the diets of some obligate frugivores[1-4]. Fruits are commonly considered as poor source of nutrition and so that the mammalian frugivores cannot achieve their energy requirements without over-ingestion. The food intake of obligate frugivores is constrained by many extrinsic and intrinsic factors and the nutritional quality of fruits may be important among them. The immediate goal of animals is to obtain sufficient energy and nutrients for maintenance, growth and reproduction[5]. Fruit choice in frugivorous bats has been studied by measuring feeding rates of several phyllostomid bats[6,7]. The pteropodid bats are known to balance their nitrogen requirements on nutritionally poor fruits but in order to do so, they are forced to over-ingest energy[8,9]. Little attention has so far been paid to find out the influence of nutritional composition

on their diet[10-12]. Frugivorous species of the paleotropical family Pteropodidae rely on nutrition poor fruits and they do not supplement insects to their diet[8]. Thereby, the obligate frugivores must rely on fruits only to meet out their nitrogen requirements.

The short-nosed fruit bat, *Cynopterus sphinx* (Vahl 1797) is an ubiquitous frugivorous bat widely distributed in India and Southeast Asia. Male ($\bar{X}$ = 48.3 ± 3.0 g) bats are comparatively larger than females ($\bar{X}$ = 45.3 ± 2.8 g). *C. sphinx* roosts alone or in small groups in "tents". *C. sphinx* also roosts in bell shaped cavity in the pendulous flower or fruit cluster of kitul palm *Caryota urens*[13]. The tent was created by bats by chewing and dropping the central flower or fruit strings and roosts under the vines of creeper plant *Vernonia scandens*[14-16]. *C. sphinx* feeds on fruits from at least 23 plant species, leaves from eight species and flower from two species[17].

The variation on the diet choice of frugivores raise a question : Does the nitrogen content of the diet of *C. sphinx* influence their food intake and hence energy intake? If so, they should over-ingest low energy and low nitrogen fruits than the energy and nitrogen rich fruits to satisfy their nitrogen requirements. This study documents the voluntary food intake and factors that influence the diet choice of *C. sphinx*.

Materials and Methods

Food choice experiments were carried out between July, 1998 and February 1999 using *C. sphinx* in captivity. Bats were captured at the Madurai Kamaraj University botanical garden using mist nets (AVINET). For each feeding trial, we confined nine *C. sphinx* for nine subsequent days. Each bat was kept under 12 : 12 h light dark cycle in a 1.0 x 0.5 x 0.5 m nylon mesh cage in which a polyethylene sheet spread over at the bottom to collect discarded materials, bolus and stools. All the polyethylene sheets were washed and fresh sheets were provided daily. Bats were fed with four different varieties of fruits, such as papaya *Carica papaya*, guava *Psidium guajava*, sapota *Achras sapota* and banana *Musa paradisiaca*, during dark hours. Every day fruits were weighed (ca. 200 g) and provided in plastic bowls at 1800 h. The discarded materials, bolus and faeces were scraped and removed from the cage around 0800 h of the next day morning and weighed by using a spring balance to the nearest 0.5 g. Daily food intake was calculated by subtracting the mass of the fruit lost in the cage from the total quantity of fruit provided initially. The nightly desiccation losses were corrected from a controlled sample of the respective night. The differences on fruit intake and preferences of one fruit to another were analyzed with one way ANOVA. The results of fruit intakes were interpreted with the nutritive values of the respective fruits, which have drawn from Gopalan *et al.*[18]

Results

Generally, the captive *C. sphinx* started to feed about 60 min after offering fruits to them. Bats plucked a piece of fruit, carried to the roosting place, masticated the fruits, extracted the juice, and ingested the fluids. After 20–30 min they started defecating the loose or mucous stools. Nightly food intake ranged from 60% to 140% of the body mass of *C. sphinx* and the intake showed significant difference ($F_{3,323} = 29.8$, $p < 0.05$). By the nightly food intake the captive *C. sphinx* gained mean quantity of 88.7 kJ, 126 kJ, 112.9 kJ and 118.5 kJ of energy from papaya, guava, sapota and banana, respectively (Table 1) and the energy intakes showed significant difference ($F_{3,323} = 5.1$, $p < 0.05$). Similarly bats ingested a mean quantity of 4.7 g, 6.6 g, 6.1 g and 6.6 g of carbohydrate from the mean intake of papaya, guava, sapota and banana, respectively (Table 1) and there was a significant difference on the intakes of carbohydrate ($F_{3,323} = 18.13$, $p < 0.05$). Bats gained 2.5 g, 3.3g, 2.5g and 1.9 g of nitrogen from the mean intake of papaya, guava, sapota and banana, respectively but there was no difference in the intakes of nitrogen ($F_{3,323} = 1.06$, $p = 0.371$).

Bats ingested high quantity of energy and nitrogen poor fruits such as papaya and guava but they also ingested least quantity of energy and nitrogen rich fruits such as sapota and banana (Table 1). The feeding response of *C. sphinx* to the four diets clearly showed that the total amount of fruit ingested daily was influenced by positive nitrogen balance. The poor energy and nitrogen diets (papaya and guava) were highly exploited by *C. sphinx* and expressed a compensatory increase in fruit intake, thereby maintaining their nitrogen requirements. Such a compensatory intake occurred only in nitrogen poor diets (papaya and guava). They did not consume excess quantities of nitrogen rich fruits (sapota and banana).

Discussion

The results strikingly show that the pteropodid bats could rely on low-protein or nitrogen and low-energy fruits. Food consumption of *C. sphinx* appears to be sensitive to the nitrogen and energy content of their diets. All the evidence seems to indicate that the *C. sphinx* ingesting high quantity of energy poor and low nitrogen fruits and least quantity of energy and nitrogen rich fruits which could supply an equal quantity of nutrition.

Table 1– Mean daily intake of energy, carbohydrate and nitrogen from *C. papaya, P. guajava, A. sapota* and *M. paradisiaca,* by *C. sphinx.*

Fruit	No. of bats tested	Quantity of fruit intake (g)	Mean daily intake			Nutritive value*		
			Energy (KJ)	Carbohydrate (mg/g)	Nitrogen (g)	Energy (KJ)	Nitrogen (g)	Carbohydrate (mg/g)
C. papaya (n=6)	9	66.2±22.9	88.7±30.7	4.7±1.6	2.4±0.9	1.3	6.0	72
P. guajava (n=6)	9	59.3±7.2	126.3±15.5	6.6±0.8	3.3±0.4	2.1	8.9	112
A. sapota (n=9)	9	25.8±9.8	112.9±42.7	6.0±2.3	2.5±1.0	4.3	16.0	235
M. paradisiaca (n=9)	9	24.4±8.2	118.5±39.8	6.6±2.2	1.9±0.7	4.8	12.9	272

*Source drawn from Gopalan *et al.*[18].

The pteropodid bats such as *Micropteropus pussilus* and *Epomops buettikoferi* could adjust the protein requirements, even their diets have 3.3% of protein[3]. The diet of herbivores and granivores consists of 7% of protein and ungulates and primates require a minimum of 7% to 10% protein in their feed to maintain positive nitrogen balance[19-21]. The daily food intake exceeding body mass appears to be the rule for frugivorous bats[8]. The protein requirements of *C. sphinx* is apparently compensated by rapid food transit time and total bulk of food that can be processed daily. This facilitates the bats to ingest up to 2.43 times (personal observation) of their body mass. The obligate frugivorous bat *C. sphinx* over ingests by rapid transit times because they reject most of the fibrous portions of the fruit prior to ingestion. Thus the pteropodids escaped from this constraint in part by reducing fiber content of ingested materials[8]. Frugivorous species of the Paleotropical family Pteropodidae also rely on nutrient poor fruits but apparently do not supplement insect diet[8]. Our observations on food intake by *C. sphinx* showed that a near 2.43 times of their body mass during lactation support the over-ingestion in pteropodid bats. Data on *Lasiurus cinereus*[22], and *Eptesicus nilssoni*[23] further support our observations. All the above evidence showed the foraging behaviour of *C. sphinx* mainly constrained by the extrinsic factor–chemical composition of their diet. The feeding response of *C. sphinx* clearly showed that the total amount of fruit ingested daily was influenced by positive nitrogen balance.

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References

1. Roberts, P. & Seabrook, W.A. (1989) *J. Zool. (Lond.)* **218** : 332.
2. Parry-Jones, K. & Augee, M.L. (1992) *Bat Res. News* **33** : 9.
3. Kunz, T.H. & Ingalls, K.A. (1994) *Funct. Ecol.* **8** : 665.
4. Kunz, T.H. & Diaz, C.A. (1995) *Biotropica* **27** : 106
5. Rasweiler, J.J.I.V. (1977) in *Biology of bats*. Vol. III. Ed., Wimsatt, W.A., Academic Press. New York, p. 519.
6. Bonaccoroso, F.J. & Gush, T.J. (1987) *J Anim. Ecol.* **56** : 907.
7. Stashko, E.R. (1992) *Ph. D. thesis*, Northwestern University.
8. Thomas, D.W. (1984) *Physiol. Zool.* **57** : 457.

9. Steller, D.D. (1986) *Aust. J. Zool.* **34** : 339.
10. Howe, J.F. & Estabrook, G.F. (1977) *Amer. Nat.* **111** : 817.
11. WheelWright, N.T. & Orians, G.H. (1982) *Amer. Natur* **119** : 402.
12. Delorme, D.W. & Thomas, D.W. (1996) *J Comp. Physiol B.* **166** : 427.
13. Bhat, H.R. & Kunz, T.H. (1995) *J Zool. (Lond.)* **234** : 597.
14. Balasingh, J. Isaac, S.S. & Subbaraj, R. (1993) *Curr. Sci.* **65** : 418.
15. Balasingh, J., John, Koilraj, A. & Kunz, T.H. (1995) *Ethology* **100** : 210.
16. Marimuthu, G., Rajan, K.E., John Koilraj, A., Isaac, S.S. & Balasingh, J. (1998) *Biotropica* **30** : 321.
17. Bhat, H.R. (1994) *Mammalia* **58** : 363.
18. Gopalan, C., Rama Sastri, B.V. & Balasubramanian, S.C. (1980) *National Institute of Nutrition.* ICMR, Hyderabad, India, p. 177.
19. Holter, J.B., Hayes, H.H. & Smith, S.H. (1979) J. *Wildlife Manage.* **43** : 872.
20. Milton, K. (1979) *Amer. Natur.* **114** : 362.
21. Minson, D.J. & Milford, R. (1967) *Aust. J. Exp. Anim. Husb.* **7** : 546.
22. Barclay, R.M.R. (1989) *Behav. Ecol. Sociobiol.* **24** : 31.
23. Rydell, J. (1991) *Ph. D. thesis*, University of Lund. Sweden.

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Laboratory rearing of *Creatonotus gangis* (Lepidoptera : Noctuidae) on artificial diet

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Abstract

An attempt was made to formulate artificial diet of *Creatonotus gangis* with 18 stored grain commodities. Larval development was quicker on triticale, wheat, pea and sorghum based diets while it was considerably prolonged on Bengal gram, finger millet, pearl millet or soybean based diets. Larval feeding on diets prepared with black gram, cowpea, French bean, green gram, groundnut, lentil, red gram or winged bean resulted in complete mortality of larvae. Higher number of adults emerged on pea, triticale or wheat based diets but larger number of normal adults were recorded on triticale based diet. Growth index and Success index also indicated that this diet was more suitable for the development of this insect

(**Keywords :** *Creatonotus gangis* / artificial diet / nutrition / rearing)

Introduction

Creatonotus gangis (L.) is a polyphagous insect[1,2]. The biology of this insect was investigated by Baser and Kushwaha[3]. However, for developing an ecofriendly insect pest management programme it is essential to formulate an artificial diet as this will help in mass rearing of *C. gangis* Such diets will also provide uniform population, even in off seasons, for conducting a variety of studies. In addition, various chemicals can be incorporated in the diet to understand their effect on the biology of the insect[4,5]. Therefore, an attempt was made to develop an artificial diet of this insect using common stored grain commodities.

Materials and Methods

A nucleus culture of *C. gangis* was maintained on soybean leaves. The adults obtained from this culture were allowed for egg laying and newly hatched larvae (0 – 12 hr old) were used for conducting the experiment. The basic composition of the diet was : Commodity (soaked) 17.67 g; yeast powder, 3.07g; sodium ascorbate, 0.31g; methyl-p-hydroxybenzoate, 0.31g; sorbic acid, 0.15g; agar, 1.54g; formaldehyde

(10%) 0.15 ml and water, 76.80 ml. Stored grain commodities used were finger millet (*Eleusine coracana*), maize (*Zea mays*), pearl millet (*Pennisetum typhoides*), rice (*Oryza sativa*), sorghum (*Sorghum vulgare*), triticale (*Triticosecale*), wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum*), black gram (*Vigna mungo*), cowpea (*Vigna unguiculata*), French bean (*Phaseolus vulgaris*), green gram (*Vigna radiata*), lentil (*Lens esculenta*), pea (*Pisum sativum*), red gram (*Cajanus cajan*), soybean (*Glycine max*), winged bean (*Psophocarpus tetragonolobus*) and groundnut (*Arachis hypogaea*). These commodities were soaked in water for 24 hr prior to the preparation of the diet. Method for compounding the diet and rearing on various diets was same as described by Bhattacharya[6]. All experiments were conducted at 29±1°C, 80 per cent r.h. and 12 hr light : 12 hr dark photoperiod. A small amount of diet was placed at the bottom of a plastic vial (6 × 25 cm) and 0-12 hr old newly emerged larvae were reared on the diet. Each treatment was replicated three times and ten larvae were used in each replication. Growth index[7] and Success index[6] were used for comparing the diets. Data were analysed with the help of a computer.

Results and Discussion

Larval survival on 4 DAF was same as was recorded on soybean leaves. Heavy larval mortality was recorded on 24 days after feeding (DAF) on red gram or groundnut based diets. During this period larval survival on Bengal gram, black gram, cowpea, finger millet, green gram, lentil, maize, rice, sorghum or soybean based diets was 70 to 90 per cent which was same as recorded on soybean leaves (Table 1). Larval survival during this period on French bean and pearlmillet based diets were 60 and 66.7 per cent, respectively. All larvae feeding on wheat or triticlae based diets completed development on 24 DAF. However, all larvae died on 8 DAF on winged bean based diet while on black gram, cowpea, French bean, green gram, groundnut, lentil and red gram based diets no larval survival was recorded after 24 DAF. The larval weight on triticale based diet on 8 DAF was same as was recorded on soybean leaves and it was significantly higher as compared to other test diets. However, low larval weight was recorded on groundnut, pearl millet or winged bean based diets. On 16 DAF significantly heavier larvae were recorded on titicale or wheat based diets as compared to soybean leaves and other test diets indicating their superiority in supporting the growth of *C. gangis*. Larval weight on pea based diet was similar to soybean leaves on 16 and 24 DAF. During this period larval weight was significantly low on diet prepared with Bengal gram, black gram, millet, French bean, green gram, groundnut, lentil, pearl millet or red gram which indicated their unsuitability for this insect.

Table 1–Survival of larvae at four days interval and weight gain by larvae of *C. gangis* on diets prepared with soaked form of different stored commodities.

Commodity	Larval survival (%)						Larval weight (mg)		
	Days after feeding						Days after feeding		
	4	8	12	16	20	24	8	16	24
Bengal gram	90.0 (74.9)	90.0 (74.9)	86.7 (68.9)	86.7 (68.9)	76.7 (65.9)	76.7 (65.9)	2.8	12.8	43.8
Black gram	100.0 (90.0)	100.0 (90.0)	90.0 (71.6)	86.7 (68.9)	83.3 (66.6)	80.0 (64.6)	2.6	5.1	8.1
Cowpea	100.0 (90.0)	90.0 (74.9)	83.3 (70.1)	70.0 (57.7)	70.0 (57.7)	70.0 (57.7)	6.3	37.9	85.5
Finger millet	100.0 (90.0)	100.0 (90.0)	76.7 (66.1)	73.3(63.9)	70.0 (57.7)	70.0 (57.7)	5.2	15.9	40.1
French bean	100.0 (90.0)	93.3 (81.1)	86.7 (68.9)	83.3 (66.1)	73.3 (59.0)	60.0 (50.9)	2.8	6.9	12.2
Green gram	100.0 (90.0)	96.7 (83.9)	90.0 (74.9)	90.0 (74.9)	86.7 (68.9)	83.3 (66.1)	3.2	6.6	11.5
Groundnut	100.0 (90.0)	53.3 (46.9)	23.3 (28.8)	23.3 (28.8)	20.0 (26.6)	16.7 (23.9)	0.5	3.0	5.6
Lentil	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	90.0 (74.9)	90.0 (74.9)	3.4	14.4	26.2
Maize	100.0 (90.0)	90.0 (78.9)	86.7 (72.8)	76.7 (62.9)	73.3 (59.9)	73.3 (59.9	2.6	38.8	207.3
Pea	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	96.7 (83.9)	10.7	183.5	658.8
Pearl millet	90.0 (74.9)	76.7 (65.9)	66.7 (59.9)	66.7 (59.9)	66.7 (59.9)	66.7 (59.9)	0.7	5.7	49.7
Red gram	96.7 (83.9)	93.3 (81.1)	50.0 (44.7)	43.3 (40.7)	36.7 (36.1)	10.0 (18.4)	2.5	2.8	2.3
Rice	96.7 (83.9)	96.7 (83.9)	93.3 (81.1)	86.7 (72.8)	86.7 (72.8)	86.7 (72.8)	5.1	48.0	203.8
Sorghum	96.7 (83.9)	93.3 (81.1)	83.3 (66.1)	83.3 (66.1)	83.3 (66.1)	83.3 (66.1)	10.0	143.3	466.5
Soybean	93.3 (81.1)	93.3 (81.1)	80.0 (67.9)	76.7 (61.7)	76.7 (61.7)	73.3 (59.9)	4.8	30.8	96.9

Table 1 Continued

Triticale	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	96.7 983.9)	*	16.1	267.8	*
Wheat	100.0 (90.0)	100.0 (90.0)	90.0 (78.9)	90.0 (78.9)	90.0 (78.9)	*	9.9	298.9	*
Winged bean	100.0 (90.0)	76.7 (65.9)	-	-	-	-	0.5	-	-
Soyabean leaf	90.0 (71.6)	90.0 (71.6)	90.0 (71.6)	90.0 (71.6)	90.0 (71.6)	90.0 (71.6)*	16.4	183.5.	694.4*
C.D. (p=0.05)	N.S.	16.5 (19.7)	23.4 (22.3)	22.7 (20.1)	28.1 (23.2)	26.7 (22.9)	1.9	26.3	75.7

Data in parentheses indicate angular transformed values

* All or some of the larvae entered pupal stage

\- All larvae died

Table 2– Developmental behaviour of *C. gangis* on diets prepared with soaked form of different stored commodities.

Commodity	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergency (%)	Growth index	Success index
Bengal gram	63.0	11.8	26.7 (30.8)	16.7 (23.9)	0.223 (10)	0.287 (11)
Finger millet	57.0@	9.0@	30.0 (33.20)@	10.0 (18.4)@	0.150 (11)@	0.440 (10)
Maize	33.3	7.8	60.0 (50.9)	60.0 (50.9)	1.460 (7)	0.793) (7)
Pea	25.0	8.5	90.0 (74.9)	80.0 (63.9)	2.382 (4)	1.017 (3)
Pearl millet	45.1	7.2	60.0 (51.9)	56.7 (49.2)	1.073 (8)	0.763 (8)
Rice	38.7	8.8	76.7 (61.7)	76.7 (61.7)	1.633 (5)	0.853 (6)
Sorghum	29.6	7.8	83.3 (66.1)	83.3 (66.1)	1.580 (6)	0.967 (5)
Soybean	43.2@	8.9@	20.0 (26.6)@	20.0 (26.5)@	0.390 (9)@	0.472(5)@
Triticale	24.9	7.1	96.7 (83.9)	96.7 (83.9)	3.023 (1)	1.110 (1)
Wheat	23.9	7.8	90.0 (74.9)	86.7 (72.8)	2.733 (2)	1.053 (2)
Soybean leaf	25.8	7.2	83.3 (66.1)	80.0 (63.9)	2.420	1.000 (4)
CD (p=0.05)	5.0	1.7	22.6 (19.5)	19.8 (16.3)		

Data in parentheses indicate angular transformed values except in growth and success indices in which it indicates ranking
@ not considered for analysis due to complete mortality in one replicate.

Table 3 - Nature of adults of *C. gangis* on artificial diets prepared with soaked form of stored grain commodities

Commodities used in the diet	Nature of adults (%)				
	Normal adults	Adults with wavy wings	Adults with undeveloped wings	Adults which failed to come out of pupal case	Incomplete eclosion
Bengal gram	0.0	6.7	0.0	6.7	
Finger millet	3.3	0.0	0.0	0.0	0.0
Maize	0.0	46.7	0.0	13.3	0.0
Pea	36.7	43.3	0.0	0.0	0.0
Pearl millet	50.3	3.3	0.0	0.0	0.0
Rice	0.0	13.3	0.0	53.3	10.0
Sorghum	16.7	66.7	0.0	0.0	0.0
Soybean	10.0	3.3	0.0	0.0	0.0
Triticale	66.7	30.3	0.0	0.0	0.0
Wheat	36.7	46.7	3.3	0.0	0.0
Soybean leaf	80.0	0.0	0.0	0.0	0.0

Table 2 indicates that quicker larval development was possible on pea, sorghum, triticale or wheat based diets indicating their superiorities as compared to other test diets. On maize or rice based diets larval period was somewhat prolonged while significant prolongation of larval period was recorded on diets prepared with Bengal gram, finger millet, pearl millet and soybean. Larger number of larvae pupated on diets prepared with pea, sorghum, triticale or wheat which was similar to soybean leaves. Pupation on rice, pearl millet or maize based diets was also not different as compared to soybean leaves. On the other hand pupation was low on diets prepared with Bengal gram, finger millet or soybean indicating their unsuitability for this insect. Pupae on all test diets except Bengal gram or finger millet based diets completed development between 7.1 to 8.9 days which was also similar to soybean leaves. Pupae formed on wheat or triticale based diets resulted in 86.7 to 96.7 per cent adult emergence, respectively. The adult emergence on remaining diets except Bengal gram, finger millet or soybean based diets was 56.7 to 83.3 per cent while it was 10 to 20 per cent on Bengal gram, finger millet or soybean based diets. Adults obtained from various diets also showed the formation of normal and abnormal adults. They were adult which normal wings, adult with wavy wings, adult with undeveloped or unstretched wings, adult which failed to come out of pupal case and incomplete eclosion. However, none of the test diets supported the formation of all normal adults (Table 3). The frequency of formation of normal adult was higher on triticale based diet. Therefore, it can be categorized as a suitable diet for this insect. Success index was more than 1.000 on diets prepared with pea, triticale or wheat. However, a Success index of 0.967 was recorded on sorghum based diet while in other diets it was less than 0.853 indicating their unsuitability for this insect (Table 2). Growth index also showed similar trend. Although pea, triticale or wheat based diets can be categorized as superior diets for growth and development of this insect but among these, only triticale based diet appears to be highly suitable as larger proportion of adults emerged on this diet were normal in nature. Earlier observation on *Spilosoma obliqua* indicated that soaked form of rice in the diet was highly suitable for this insect while soaked form of soybean or wheat in the diet showed a little prolongation of larval period.[9].

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References

1. Catindig, J.I.A., Barrion, A.T. & Litsinger, J.A. (1993) *Intl. Rice. Res. Notes.* **18 :** 34.
2. Jamwal, S.S. & Bhattacharya, A.K. (2000) *Host range of Creatonotus gangis* (Lepidoptera : Arctiidae). Unpublished.
3. Baser, S.L. & Kushwaha, K.S. (1968) *Univ. Udaipur Res. Studies.* **6 :** 124.
4. Yang, W.H., Ma, L.H., Zhu. H.Q. & Xiang, S.K. (1999) *Acta Gossypii Sinica* **11 :** 31.
5. Cook , S.P., Webb, R.F. & Thorpe, K.W. (1996) *Env. Ent.* **25 :** 1209.
6. Bhattacharya, A.K. (1993) *Reseach Bulletin No. 112.*, G.B. Pant Unversity of Agriculture and Technology, Pantnager-263 145, India.
7. Pant, N.C. (1956) *Indian J. Ent* **73 :** 106.
8. Prasad, J. & Bhattacharya, A.K. (1975) *Z. Ang. Entomol.* **79 :** 34.
9. Tiwari, S.N. & Bhattacharya, A.K. (1987) *Memoir No. 12, Memoirs of the Entomological Society of India.* The Entomological Society of India, Division of Entomology, I.A.R.I., New Delhi, India.

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Efficacy of distillery effluent on seed germination and seedling growth in mustard, cauliflower and radish

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Abstract

A laboratory experiment was conducted to study the effects of different concentrations (5-100%) of distillery effluent on germination, speed of germination, peak value, germination value and seedling growth of different crop species i.e., mustard, cauliflower and radish. In all the three crops, no germination was observed at 75 and 100% concentrations. Radish recorded the highest germination percentage (100%) in control followed by cauliflower (98%) and (94%). In radish and cauliflower germination percentage was at par with control up to 25% concentration among the treatments. In mustard on the other hand, germination percentage declined significantly from 25% on wards. Similar trend was observed with speed of germination, peak value and germination value. Root length and hypocotyl lengths were highest at 5% effluent and in subsequent treatments declined. In cauliflower, the dry weight increased with increase in the concentration up to 20% and declined later. The results suggest that, radish and cauliflower were more tolerant to distillery effluent than mustard and it should be diluted properly to avoid harmful effects.

(**Keywords :** distillery effluent/germination/mustard/cauliflower/radish).

Introduction

Rapid industrialisation in India has resulted in the substantial increase in the liquid waste (Spent wash or effluent) which is traditionally discharged in open land or into nearby natural water causing a number of environmental problems including threat to plants and animal lives and also creating problems in surface water logging, ground water contamination and salinizing good quality land due to presence of high quality salt contents. But, it is very rich in organic carbon, potassium and moderate levels of nitrogen[2,6,10]. At present, most of it is discharged in natural water causing widespread damage to aquatic life. However, if it is treated properly before discharge it may not

cause pollution in the atmosphere and can safely be used for irrigation purposes. If the toxins in the effluent are beyond the optimum tolerance limits it may cause irreparable damage. Hence, the present experiment was conducted to find out the effect of the spent wash (distillery effluent) on seed germination and seedling growth of mustard, cauliflower and radish.

Materials and Methods

Twenty five seeds of each crop species namely mustard (*Brassica junea* Var. Pusa Bold) radish (*Raphanus sativum* Var. Varsha Karani) and caulifloer (*Brassica oleracea* Var. P.D.) wer spread uniformly in sterlised petridishes of uniform size having moistened Whatman No. 1 filter paper. The filter paper was moistened with 2 ml of distilled water for control and with same quantity of various concentrations of the effluent (5, 10, 15, 20, 25, 50, 75 and 100%) diluted with distilled water and was kept at room temperature for germination studies. The entire experiment was conducted three times with four replications in each treatment including control for confirmation of the results. Observations on the number of seeds germinated were recorded daily until there was no further germination. Germinated seed were allowed to grow for one week to record observations on root length, hypocotyl and dry weight. Finally, data were computed for speed of germination,[4,11] peak value[1] and germination value[1] using the following formulae :

$$\text{Speed of germination} = \frac{\text{Number of seeds germinated}}{\text{Days of first count}} + \ldots\ldots + \frac{\text{Number of seeds germinated}}{\text{Days of final count}}$$

Peak value = Highest quotient obtained by dividing the cumulative percent germination on each day by number of days elapsed since initial imbibition

Germination value = Peak value X Germination (%).

Results and Discussion

In the present investigation, data on pH, electrical conductivity (E.C.) and osmotic pressure (OP) in the concentrated effluent and its various levels of dilutions showed that, absolutely there was no difference in pH in any dilution of the effluent but E.C. and O.P. were affected highly and increased with concentration of the effluent (Table 2). Among the seeds of three crops studied, radish recorded the highest germination percentage (100%) up to 15% concentration of the effluent and declined slightly (98%) at 20% and subsequently 80% at 25% concentrations. Even at 50% concentration also, the germination percent was > 50% (66%). In cauliflower, the germination was more or less stable up to 25% and decreased sharply from 50% onwards. On the other hand, in mustard, there was a gradual reduction right from 5% onwards and declined sharply after 20% concentration (Fig. 1). Lower concentration of effluent increased the germination percentage in comparison to higher concentration, but was at par with control. Our results are in conformity with the findings of Thamizhiniyan *et al*[11]. However, with increase in the concentration of the effluent, there was a significant reduction in the percent germination in all the three crop species with practically no germination at 75 and 100% concentration. The reason for the reduction in germination percent at higher concentration might be attributed to high total solids (9.8%), excessive quantities of inorganic salts i.e. sulphate S (1.31 g L^{-1}), chloride (4.05 g L^{-1}) organic C (37.4 g L^{-1}) and high osmotic pressure (Table 1) of solution which probably affects the percentage germination and seedling growth as was also observed by Mishra and Saxena[5]. Further, it was reported that high osmotic pressures of the germination solution make imbibition more difficult and retard germination [8]. The ability of seeds to germinate under high osmotic pressures differs with variety as well as species[12]. Similar types of results were also obtained in *A. catechu* and *D. sisso*[7]. In general, each crop is having a different type of germination pattern governed by initial germination, rate of germination, seed size and changes in the rate of germination over time. Effluent had very high buffering capacity, as the pH of the solution did not change even after dilution in comparison to concentrated one. Furthermore, Justice and Reece[3] reported that germination can occur over a wide range of hydrogen ion concentrations and the germination of almost all species occurs between pH values 4.0 and 7.6. It appeared from the study that, the acidity of the effluent has not interfered with germination. In the present investigation, fungal attack was seen in higher concentrations, which confirms the findings of Pandey and Soni[7] who reported that germination decreased beyond 10% and ascribed the

reason to higher fungal attack and its toxic in nature. The toxicity might be because of the presence of phenols and was also evident from brown black coloration of the seed coat after treatment with the effluent.

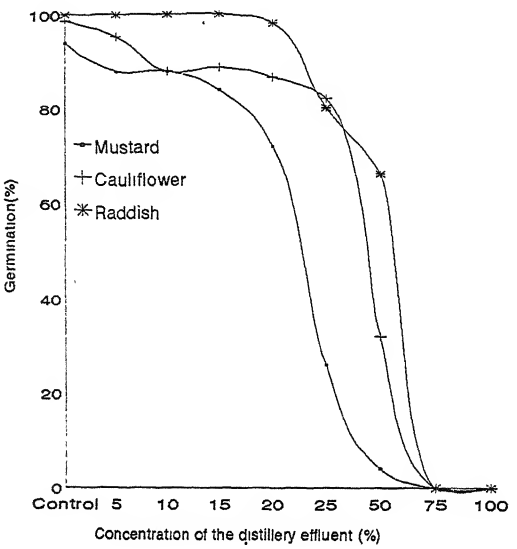

Fig. 1– Effect of distillery effluent on final germination in mustard, cauliflower and radish

Speed of germination in all the three crops was affected significantly with effluent (Table 2). The differences were more pronounced in cauliflower than in mustard and radish. In this crop, 5% effluent and control were at par and significantly superior to all other treatments. In mustard and radish, the declining trend was observed at higher concentrations (20%). A significant reduction in peak value was observed from 10% effluent in mustard and cauliflower. In radish however, the peak value declined from 20% concentration onwards. The effect of effluent on germination value was similar to peak value. There was a drastic reduction in the length of the root and hypocotyl of mustard and cauliflower in all the concentrations compared to control. Whereas, in radish, at the lowest concentration (5%) there was a significant increase followed by decrease in both the parameters. Dry weight of the mustard seedlings was not affected by the effluent but increased in cauliflower and radish with higher concentration (Table 2). Similar type of results was observed in maize crop with distillery effluent[9]. The

results suggest that, radish and cauliflower were tolerant to distillery effluent compared to mustard. However, careful consideration of the concentration of the effluent to be applied is a prerequisite before using it for presowing irrigation purposes.

Table 1– Characteristics of different concentration (%v/v) of the distillery effluent.

Effluent Concentration (%)	pH	EC (d S m^{-1})	OP[1] (atmospheres)
5	4.0	1.71	0.62
10	4.0	3.27	1.17
15	4.1	4.90	1.76
20	4.0	4.90	1.76
20	4.0	5.16	1.86
25	4.1	5.16	1.86
25	4.1	7.60	2.74
50	4.1	7.60	2.74
75	4.1	12.05	4.33
75	4.0	17.12	6.16
100	4.1	25.30	9.11

[1]OP = E.Ce X 10^3 X 0.36 (USDA, Agric. Hand Book, No. 60)

Table 2 -Effect of distillery effluent on speed of germination, PV,GV, length of root and hypocotyl (cm) and of dry weight mustard, cauliflower and radish

Effluent Concentration (%)	Speed of Germination			Peak value			Germination value			Length of radical (cm)			Length of hypocotyl (cm)			Dry weight (mg seedling $^{-1}$)		
	M	C	R	M	C	R	M	C	R	M	C	R	M	C	R	M	C	R
Control	81.7	94.0	97	70	91	94	6592	8965	9400	8.00	4.86	5.50	2.37	1.88	3.01	5.58	1.81	9.77
5	79.5	88.3	97	70	85	94	6512	8133	9400	4.13	1.48	5.85	2.40	1.81	3.15	5.05	1.31	9.65
10	62.5	77.3	97	38	72	94	3240	6336	9400	3.73	0.91	4.33	2.22	1.75	3.04	4.95	1.55	9.45
15	58.3	63.5	96	34	29	93	2928	2523	9300	2.63	0.71	3.84	1.61	1.15	2.20	5.13	1.81	10.04
20	32.7	54.7	86	7.6	21	76	560	1793	7432	0.32	0.65	2.67	0.55	0.77	1.62	2.59	3.63	10.01
25	11.3	48.2	66	2.4	22	56	67.6	1841	4512	-	0.43	2.49	-	0.64	1.15	-	2.48	10.45
50	0.5	13.3	35	0.13	10	9.8	0.50	321	668	-	-	0.39	-	-	0.27	-	-	8.05
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CD (0.05)	19.6	8.02	9.63	26.5	11. 9	13.8	2418	1350	1309	0.79	0.36	0.18	0.24	0.18	0.58	1.14	1.44	1.64

Note: M, C and R denote for mustard, cauliflower and radish respectively.

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References

1. Czabator, F.J. (1962) *For Sci.*. **8** : 386.
2. Devarajan, L & Oblisami, G. (1995) *Madras Agric. J* **82** : 664.
3. Justice, O.L. & Reece, M.H. (1954) *AOSA* **34** : 144.
4. Maguire, J.D. (1962) *Crop Sci.* **2** : 176.
5. Mishra, K. & Saxena, D.K. (1996) *paper presented in the A. Session (Biological Sciences Section) of the Nat Acad Sci. India.* (Abstract Book) p. 132.
6. Neelam & Sahai, R. (1988) *J. Envi Biol* **9** : 45.
7. Pandey, D.K. & Sony P. (1994). *Indian J For* **17 (1)** : 35.
8. Rodger R.B.B., Williams G.G. & Davis R.L. (1957) *Agronomy J.* **49** : 88.
9. Singh, Y. & Rajbahadur (1998). *Indian J Agric. Sci* 68 : 70.
10. Sweeney, D.W. & Graetz, D.A (1991). *Agri. Eco Environ.* **33** : 341.
11. Thamizhaniyan, P., Sundaramurthy, P. & Lakshmanachary, A.S. (1998) in *Abstracts of Nat. Sym. Dept. Bot,* Annamali Univ., Tamilnadu. p. 3.
12. Ungar, I.A. (1987) *Bot. Rev.* **44** : 233.

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Inter relationship amongst various physico-chemical factors, phyto and zooplankton of a lotic water body of Indo-Nepal Himalayan Terai region of Bihar

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Abstract

Intra correlation and coefficient variations amongst various physico-chemical factors, phyto and zooplankton communities of four virgin sites of Sirkrahna river of Indo-Nepal Himalayan terai region of Bihar were evaluated during spring and late rainy season in the year 1999. During biostatistical analysis of the collected data, temperature was found positively correlated with temperature, DO and total alkalinity. The positive correlation between other physico-chemical factors such as DO and phosphate, DO and pH, pH and phosphate were also obtained. The coefficient variations of phytoplankton population was found less variable than zooplankton whereas correlation coefficient value showed quite positive between both phyto and zooplankton, provides better condition for high yield of phytoplankton and aqua culture process of terai river.

(**Keywords** : Himalayan terai/Sirkrahna river/physico-chemical factors/biotic factors/correlation coefficient)

Introduction

The habitat of an organism actually represents a particular set of environmental conditions suitable for its successful growth "Ecological niche". This includes the physical space occupied by biotic masses, their functional role in the community and their position in the environmental gradients in different physico-chemical parameters. The different plankton biomass present in the ecosystem are linked by nutritional requirement in which there is transfer of food energy through a series of repeated eating and being eaten. These pyramids of plankton community are the sole products of the hydro ecosystem. Water becomes the basic element of all these agriculture and its specific properties as a culture medium are of great significance in the productivities of a water body because it is governed by physico-chemical and

biological environment. It is a very much a part of ecosphere where it is bound to be influenced by other communities organism of total biosphere. The productivity and production rate of a water body depends upon the quality of water. The importance of primary productivity studies in an aquatic environment is well realised in view of its value in estimating the productivity capacity.

The hydrobiological conditions cannot be generalised and hence each water body has to be studied to assess its production potential. Also many of the limnological studies do not take into account the influence of water in contributing to productivity of water body. Further, the inadequate knowledge of the positive and negative correlations between plankton community and various physico-chemical factors are a major handicap to a better under standing of the factors influencing the biological productivity of any fresh water body.

The Someshwar range forms the northern boundary of old Champaran distt. and Narayani or Burhi Gandak forms the western boundary. The general flow of this river if first from north to south and then from north-west to south-east, the later being the predominant course of the river. In the northern course, it is east, the later being the predominant course of the river. In the northern course, it is known as Sikrahna and the southern as the Burhi Gandak. It originates from the Chautarwa chaur near Chanpatia in the form of a comparatively narrow canal in the West champaran and passing through the investigated sites, terminates in to river Ganga near Khagaria (Bihar). It is perennial fresh water lotic body with virulent water current especially during the monsoon season. The data were obtained from four different sites stretching over about 15 km of radius to and from Sagauli area. An examination of the physico-chemical conditions of the river Sikrahana at four different centres in the year 1999 (November) indicated that the average temperature of water ranged between 19.5°C and 20.9°C. The range of various chemical parameters of these sites were recorded to be as follows :—

pH----------6.7-7.8

DO----------6.2-7.4

Turbidity-----Less than 100

Conductivity---215-430

Alkalinity--------80-210 ppm

Chloride----------4.0-8.4 ppm

Nitrate-----------4.0-8.4 ppm

Phosphate-------0.04-0.16

Calcium--------28.5-77.9 ppm

Hardness-------90-138 ppm

Taking all these into consideration, a question was formulated with an aim to investigate, evaluate and ascertain the biotic factors, physico chemical factors and inter-correlate them through various biostatistical analysis for the better understanding of the productivity of fresh water body of this terai region which is originator of many rivers and rivulets of flood prone North Bihar, India.

Materials and Methods

Standard protocols for physico-chemical analysis[2] and limnological study[3] were adopted as per standard methods. The statistical method were applied for the bio-statistical analysis of the collected data during the present investigation using standard deviation (SD), coefficient value (CV), correlation coefficient (r) and test of significance (t).

Topography : Several ghats or sites across the river have been put up by making slopes and approach roads. The present four collection centres (P_1, P_2, P_3, & P_4,) namely Sapaha ghat, Madhumalti ghat, Gorigawan ghat and Dhanahi ghat were selected for obtaining physico-chemical and plankton analysis during the investigation period. The distance of all the four sites were in 15 km radius to each other situated near N.H. 28 and on N.E. Railway (Latitude 26° 40 N, longitude 84°17 E). It is a fresh water lotic body of perennial nature having close link to the terai region of Indo-Nepal Himalayan range of Bihar (Fig-1).

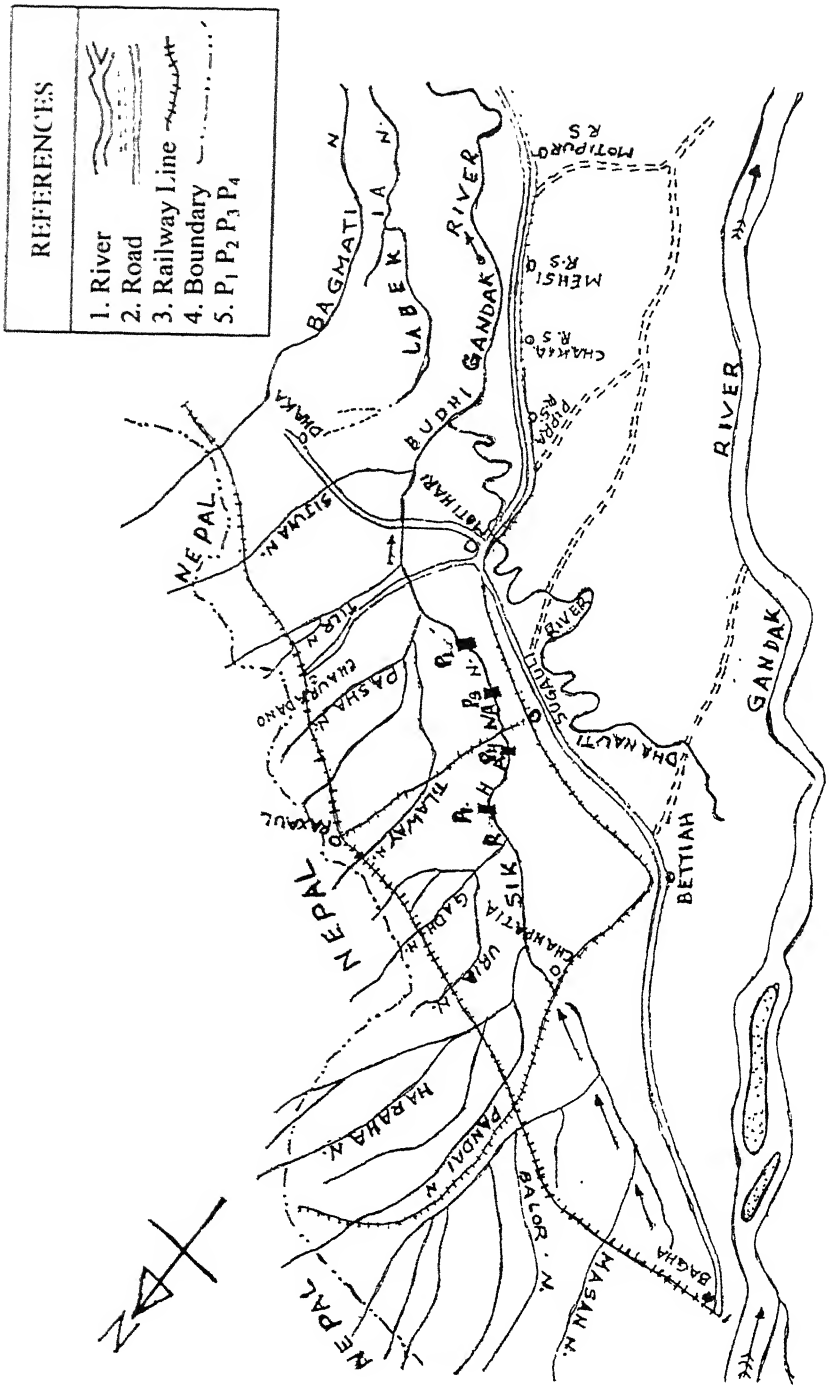

Fig 1– Showing Sikrahana river course in Indo-Nepal Himalaya Terai region depicting the investigating sites and other details.

List of important fishes found at the different sites of the Sikrahna river :—

Sl No.	Name of fish	Local name	English name
1.	*Notopterus notopterus*	Patra	Grey feather back
2.	*Satipinna phasa*	Phansi	Gangetic hair fin anchovy
3.	*Catla catla*	Bhakura	Catla
4.	*Cirrhinus mrigala*	Nainee	Mrigal
5.	*Labeo angra*	Kharsa	Angro labeo
6.	*Labeo calbasu*	Calbasu	Black rohu
7.	*Labeo rohita*	Rohu	Rohu
8.	*Labeo bata*	Bata	Bata labeo
9.	*Labeo pangusia*	Rewa	Pangusia labeo
10.	*Chela cachius*	Chelwa	Silver hatchel chela
11.	*Chela lanbuca*	Dandula	Indian glass barbs
12.	*Salmostoma baciala*	Chelhava	Large razon belly minnow
13.	*Botia dario*	Baghi	Loach
14.	*Mystus tengra*	Tengra	Tengra mystus
15.	*Mystus vittatus*	Tengra, Palwa	Striped dwarf cat fish
16.	*Xenentodon cancila*	Kauwa	Fresh water gar fish
17.	*Ompok bimaculatus*	Jal kapoor	Butter cat fish
18.	*Wallogo attu*	Buari, Brari	Fresh water sharp
19.	*Monopterus cuchia*	Bami	Cuchia fresh water eel
20.	*Chanda ranga*	Channari	Indian glass fish
21.	*Glossogobius gluris*	Bulla	Bar eyed goby
22.	*Colisa fasciatus*	Kotra	Giant gourami
23.	*Macrognathus aral*	Patya	One stripe spiny ell
24.	*Sisor rhabdophorus*	Bistulya	Sisor cat fish

25.	*Aplochilus panchax*	Dendula	Lesser tip minnow
26.	*Esomus denricus*	Dendula	Flying barb
27.	*Brachydanio terio*	Palava	Zebra fish
28.	*Nema cheilus corica*	Khorkey	Loach
29.	*Crosso chellus latius latius*	Petphorani	Gangetic latia
30.	*Garra gotyla gotyla (grey)*	Siltoka	Gotyla stone sucker

Results and Discussion

The statistical data collected during the course of investigation exhibited a varied picture of correlation between different parameters (Table 1).

Table 1– Correlation matrix between abiotic Parameters of river Sirkrahna.

	PH	DO	FCO2	PHOSPHATE	TOTAL ALKALINTY	CHLORIDE
Temperature	r=-.162	r=+.658	r=+.538	r=+.567	r=+.461	r=+.784
	t=.751	t=4.244	t=3.018	t=3.248	t=2.421	t=6.11
PH		r=-.089	r=-.121	r=+2.49	r=+.141	r=+.229
		t=.42	t=.57	t=1.20	t=.67	t=1.10
DO			r=-.451	r=+.359	r=-.258	r=+.589
			t=2.362	t=1.81	t=1.25	t=3.417
FCO_2				r=+.789	r=+.748	r=-.712
				t=6.158	t=5.315	t=4.721
PHOSPHATE					r=-.799	r=+.749
					t=6.29	t=5.33
TOTAL ALKALINITY						r=+.631
						t=3.81

N = 24

The detailed quantitative population of plankton were observed and found that chlorophyceae amongst phytoplankton and rotifers amongst zooplankton were present in maximum numbers at all the selected ghats of Sikrahna river (Table 2).

Table 2– Quantitative analysis of plankton abundance of river Sikrhna at different selected sites (per liter of water).

Phyto--Site	Chloro	Cyano	Bacillar	Eugleno	Chryso	Total	%
P1	277	102	210	28	16	633	23.91
P2	281	128	219	21	15	664	25.08
P3	272	132	206	27	20	657	24.82
P4	283	139	223	29	19	693	26.18
Total	1113	501	858	105	70	2647	

Zoopla-nkton	Copepoda	Ostracoda	Rotifera	Cladocera	Protozoa	Total	%
P1	123	50	157	67	20	417	23.33
P2	135	52	165	75	27	454	25.37
P3	134	54	154	69	27	438	24.48
P4	139	59	179	84	19	480	26.83
Total	531	215	655	295	93	1789	

Confirming earlier findings temperature was found positively correlated with pH[4] DO[3], Phosphate, Chloride[5] and negatively with FCO_2 and total alkalinity[7,8]. On the basis of observed "r" value FCO_2 was found positive correlated with phosphate and total alkalinity and negative with chloride. Its established positive correlation with total alkalinity and phosphate showed positive correlation with chloride and negative correlation with total alkalinity. Total alkalinity showed negative correlation with chloride[4]. pH recorded positive correlation with phosphate and chloride whereas it showed negative correlation with DO, FCO_2 and total alkalinity. DO, FCO_2 and total alkalinity exhibited positive correlation with themselves whereas positive between phosphate and chloride[6,8]

Table 3– Correlation matrix between abiotic and biotic parameters of Sikrahna.

ABIOTIC FACTORS	BIOTIC FACTORS	
	PHYTOPLANKTON	ZOOPLANKTON
1. Temperature	r=+0.050[6] t= 0.235	r=+0.123[4] t= 0.583
2. pH	r=+0.049 t= 0.234	r=-0.015 t= 0.74
3. DO	r=+0.179[3] t= 0.854	r=+0.254[1] t= 1.323
4. FCO_2	r=+0.012 t= 0.056	r=-0.061[6] t= 0.289
5. PHOSPHATE	r=+0.040 t= 0.191	r=+0.025 t= 0.119
6. TOTAL ALKALINITY	r=-0.103[5] t= 0.486	r=-0.098[5] t= 0.462
7. CHLOR.	r=+0.066 t= 0.031	r=+0.230[2] t= 1.109

1. Significant at 0.20 level
2. Significant at 0.30 level
3. Significant at 0.40 level N=24
4. Significant at 0.60 level
5. Significant at 0.70 level
6. Significant at 0.80 level

Table 4– Monthly variation in total zoopalankton per litre water.

Centre	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total	Mean	SD
P_1	12	17	32	49	68	32	13	12	38	76	46	22	417	34.75	11.95
P_2	11	19	23	37	63	37	17	13	11	90	79	24	454	37.83	15.19
P_3	16	29	38	61	68	59	21	18	18	61	31	18	438	36.50	11.14
P_4	13	27	33	64	82	53	17	13	12	89	58	19	480	40.00	15.51

Mean = 37.27
S.D. = 13.44
C.V. = 36.06 %

Table 5– Monthly variation in total phytoplankton per litre water.

Centre	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total	Mean	SD
P_1	08	25	39	74	107	120	74	22	14	68	71	11	633	52.75	21.13
P_2	18	23	51	71	105	103	78	29	18	81	56	31	664	55.33	18.25
P_3	15	68	46	76	108	105	83	27	19	58	74	29	657	54.75	18.71
P_4	14	26	51	79	107	115	84	25	25	69	63	35	693	57.75	18.73

Mean = 55.14
S.D. = 19.20
C.V. = 34.82 %

DO showed positive correlation with phosphate and chloride and negative with FCO_2 and total alkalinity[7,8]. FCO_2 showed positive correlation with phosphate and total alkalinity whereas it showed negative with chloride. Phosphate and total alkalinity exhibited, positive correlation between them and showed negative correlation with chloride[5,7]. Total alkalinity showed negative correlation with chloride[8].

Phytoplankton experienced positive correlation with temperature, pH, DO, FCO_2 and phosphate and negative correlation with total alkalinity and chloride[10,11,12]. As per 'r' value calculation, the correlation with phytoplankton with tem-perature (at 0.80 level), DO (at 0.40 level) and negative correlation with total alkalinity (at 0.70 level) while it was insignificant with other parameters (Table 3).

Zooplankton experienced positive correlation with temperature, DO, phosphate and chloride while negative correlation was recorded with pH, FCO_2, total alkalinity. As per 'r' value calculation, the correlation of zooplankton was found positive correlation with temperature (at 0.60 level), DO (at 0.20 level) and chloride (at 0.30 level) while negative correlated with FCO_2 (at 0.80 level) and with total alkalinity (at 0.70 level). Their correlation with other parameters was observed to be insignificant (Table-3)[14,15,19].

Coefficient of variations was calculated to determine the event of variation in both plankton (phyto and zoo) population[17]. During the present investigation value of C.V. of zooplankton (Table-4) and phytoplankton (Table-5) are recorded. Zooplank-ton (Table-4) exhibited slightly greater variation than that of phytoplankton.

The positive correlation of phytoplankton with temperature, pH, DO, FCO_2 and phosphate were found in agreement with earlier findings and negative correlation with chloride and total alkalinity was in accordance with the findings of several worker[14,16]

The positive correlation of zooplankton with the temperature, DO, phosphate and chloride in the present study was in agreement with the findings of earlier workers[13,17] whereas its negative correlation with pH, FCO_2 and total alkalinity was in the agreement with the findings of some workers[15,18,19]. The present study led to the conclusion that all these factors and findings in composite form provide better environment and conditions for high yields of phytoplankton and the potential aquaculture process of this river in this region.

References

1. Jatin, Yatish & Dhamija, S.K. (2000) *J. of Environ & Pollution* **7 (2)** : 83.
2. A.P.H.A. (1985) *Standard method for examination of water and waste water* APHA, AWWA, WPFC, 16th Ed.; New York, p. 1268.
3. Welch (1952) *Limology*, 2nd edn. Mc Grew Hills Book company, New York.
4. Chauhan, R. (1995) *Flora* and *Fauna* **1** : 77.
5. Sculthrope, C.D. (1967) *The biology of aquatic vascular plants.* Edward Arnold (Pub.) Ltd. London. p. 610.
6. Mesfin, Melaku & Belay Amha (1989) *International J. of Eco of Environ. Sci.* **15(1)** : 1-16.
7. Moyle, J.B. (1946) *Trans. Amer. Fish Soc.* **76** : 322.
8. Boyd, C.E. (1979) *Water quality in warm water fish pond.*
9. Mair D.F. (1966) *Limnol. Oceanagar* **11** : 68.
10. Dobriya, A.K. and Singh, H.R. (1981) *J. Zool.* **1** : 16.
11. Saxena, K.K. and Chauhan, R.P.S. (1993) *Poll. Res* **12(2)** : 101.
12. Joshi, S.N., Tiwari, N.C. and Gaur, M.R. (1989) *Environ. & Ecol.* **7(1)** : 105.
13. Lakshminarayan, J.S.S. (1985) *Hydrobiol.* **25** : 119.
14. Dubery, S., Kumari Dibya and Tiwari, P.B. (1989) Mendel **6(374)** : 453.
15. Kumar, K. (1990) in *Recent Trends in Limno,* p. 301.
16. Pandey, U.C. Tiwari, G.L. and Pandey, D.C. (1989) *J. Indian Bot. Soc.* **68** : 277.
17. Mahajan, B.K. (1984) *Methods in Biostatistics*, 4th edn. Pub. Indu Mahajan, Pusa Road, N Delhi p. 60.
18. Hai, L.T. (1985) *Hydrobiol* **128(2)** : 161.
19. Abdullahi, Bukar. A. (1990) *Hydrobiol* **196** : 101.

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Effect of herbal antifungal agents on 33 *Trichophyton* isolates

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Abstract

Thirty-three *Trichophyton* isolates representing different species and varieties have been examined for their inhibition by 8 test samples derived from *Ricinus communis* (castor) seed oil, *Cocos nucifera* (coconut oil), *Azadirachta indica* (neem) seed oil. *Salicornia* seed oil and extracts of *Oscimum sanctum* (tulsi) leaf, neem leaf and *Allium sativum* (garlic) scales and cloves. The results, based on minimal inhibitory concentration (MIC), suggest that except 2 samples (coconut oil and garlic clove extract) rest of the samples caused complete inhibition at varying concentrations. Garlic scales and tulsi leaf extracts showed highest antifungal activity against most *Trichophyton* isolates(16 and 2 isolates, respectively), followed by neem seed oil. The garlic clove was ineffective as an antifungal compound. Similarly, the neem leaf extract had only feeble toxicity with most isolates.

(**Keywords :** dermatophyes/*Trichophyton*/herbal medicines/antifungal compounds)

Introduction

The widespread occurrence of dermatophytic infections and the limited number of available, drugs[1-3], with 'worse than the disease' side effects[4,5], have necessitated the search for herbal medicines derived from the plant-kingdom. There is a global search for safe antifungal compounds and much information has recently been made available regarding antifungal activities of plant-derived chemicals[6-12].

In the present study we have examined 33 *Trichophyton* isolates representing different species and varieties : *T. mentagrophytes* (12), *T. raubitschekii* (1), *T. rubrum* (20) for their inhibition by samples derived from plant sources viz. *Ricinus communis* (castor) seed oil, *Cocos nucifera* (coconut) oil, *Azardirachta indica* (neem) seed oil, *Salicornia* seed oil and extracts of *Oscimum sanctum* (tulsi) leaf, neem leaf, and *Allium sativum* (garlic) scales and cloves.

Materials and Methods

The Test Organisms : The dermatophytic samples were collected from hair, nail and skin of patients from local hospitals and identified by the Kane/Fischer system of classification[13] based on their growth patterns on 9 diagnostic media. Thirty-three isolates obtained from hari (7), nail (6), and skin (2) used in this study belonged to the following species and varieties of *Trichophyton.*

Trichophyton mentagrophytes (cottony form)	:	5 isolates (hair 3, nail 1, skin 1)
T. mentagrophytes (granular form)	:	6 isolates (hair 2, nail 2 skin 2)
T. mentagrophytes (velvety form)	:	1 isolate (hair 1)
T. raubitschekii	:	1 isolate (hair 1)
T. rubrum var granular (granularum)	:	1 isolate (skin 1)
T. rubrum var melanoid (nigricans)	:	2 isolates (skin 2)
T. rubrum var *rubrum*	:	14 isolates (nail 3, hari 11)
T. rubrum var velvety (velutinum)	:	3 isolates (skin 3)

Inoculum Preparation[14] : The organisms were grown on Sabouraud dextrose agar[15] for 7 days. Five colonies of 1 mm diameter were taken in 5ml of 0.85% sterile saline. The turbidity of conidial cell suspension was measured at 530nm and adjusted with sterile saline to match 0.5 Mc Farland standard to give a suspension of 1×10^6 to 5×10^6 conidia /ml.

Preparation of Plant Test Samples :

1. Oils from seeds (neem, castor, *Salicornia* and coconut)

A 100 g of powdered seed sample with hexane was used for extraction in a soxhlet apparatus for about 12 hr. Solvent was distilled off and the extract was evaporated to obtain the oil (100%).

2 Preparation of water extracts of leaves of neem, tulsi, garlic (scales & cloves)

Crushed leaves (50 g) of neem, tulsi and scales and cloves of garlic were boiled in distilled water (1:2 w/v) for 5 min and the filtrate used as test compound.

Macrobroth Dilution Susceptibility Test for Minimal Inhibitory Concentration (MIC)[14] : Twelve sterile tubes were taken and 2 ml of Sabouraud dextrose broth were added to each. An aliquot of 2 ml of plant extract (100%) was added to tubes 1 and 2; 2 ml from tube 2 was added to tube 3. Serial dilution was carried out by transferring 2 ml from each tube, using fresh pipette for each dilution and discarding 2 ml from the last tube. This gave a concentration of 100% to 0.05%. From each of the 12 tubes, 0.5 ml aloiquot was removed and 0.1 ml distributed into 4 sterile tubes. Out of the 4 tubes, 2 tubes were inoculated with 0.9 ml of standardised inoculum, which acted as test and the remaining 2 with 0.9 ml sterile saline which served as 'control'. The remaining 1.5 ml from the dilution was kept as control of any contamination.

Eight sterile tubes were taken and 0.1 ml of drug-free broth was added to each of the tube. An aliquot of 0.2 ml of 10% dimethyl sulphoxide (DMSO) was added to the tubes (4 with and 4 without DMSO) for use as 'growth control'. All the tubes were incubated at 27 °C for 48 to 72 hr. and were visually read for turbidity. Growth in each tube was scored as indicated below[16] :

4+	=	abundant growth;
3+	=	approximately 25% reduction in growth;
2+	=	approximately 50% reduction in growth;
1+	=	75% reduction in growth;
0	=	No growth.

The lowest concentration, which showed 1+ grwoth in the test was taken as the MIC as suggested by Hazen[16]. The understanding of MIC varies with the method used. Typically, the azoles are considered fungistatic and give some degree of trailing in broth dilution assays. For this reason, the MIC in macrobroth dilution assays is usually taken as the lowest concentration giving 1+ growth (MIC-1)[17].

Results and Discussion

Table 1 shows the MIC (%) values of the test plant samples that arrested growth of the various isolates of *Trichophyton* spp. It is interesting to note that coconut oil and extract of garlic cloves had no inhibitory effect on any of the 33 test fungi, while others which caused complete inhibition (tubes which showed 1+ growth; Hazen[17]) varied in their inhibitory potential. Most inhibitory samples were derived from garlic

scale and tulsi leaf followed by neem seed oil. The antifungal activity was localized in certain parts of the plant. In garlic the scale was the most fungitoxic and the clove, totally ineffective. This was also the case with neem; the seed had antifungal activity equal to that of garlic scales, but the leaf extract generally exhibited only a feeble toxicity, exception being SD 18 of *T. mentagrophytes* (cottony form), SD 16 *T. mentagrophy-tes* (velvety form). Tulsi leaf extract brought about maximum inhibition among the test plant samples for *T. raubitschekii,* SD 2 or *T. mentagrophytes* (granular form) and SD,19 *T. rubrum* var granular (granularam).

Table 1– Showing effect of plant extracts on 33 isolates of *Trichophyton* species.

Organisms		MIC (%)							
		Castor seed oil	Coconut oil	Neem seed oil	*Salicornia* seed oil	Tulsi leaf	Garlic scales	Garlic clove	Neem leaf
T. mentagrophytes (cottony form)	SD 3	25	0	25	50	50	25	0	25
	SD 7	50	0	25	25	50	12.5	0	25
	SD 18	50	0	50	25	50	6.25	0	25
	SD 21	50	0	25	50	50	25	0	50
	SD 22	50	0	25	25	25	12.5	0	25
T. mentagrophytes (granular form)	SD 2	25	0	25	50	12.5	12.5	0	50
	SD 10	25	0	6.25	12.5	50	6.25	0	25
	SD 20	25	0	12.5	50	50	6.25	0	25
	SD 24	25	0	25	50	50	6.25	0	25
	SD 27	50	0	25	50	25	12.5	0	25
	SD 33	50	0	6.25	25	50	12.5	0	50
T. mentagrophytes (velvety form)	SD 16	50	0	25	50	50	6.25	0	12.5
T. raubitschekii	SD 8	50	0	50	50	12.5	25	0	25

Table 1 Continued

T rubrum var granular (granularum)	SD 19	50	0	50	50	25	25	0	25
T rubrum var melanoid (nigricans)	SD 6	25	0	50	25	50	25	0	50
	SD 15	25	0	6.25	25	50	25	0	50
T. rubrum var *rubrum*	SD 1	50	0	12.5	50	50	12.5	0	50
	SD 5	25	0	25	25	25	6.25	0	50
	SD 11	12.5	0	25	12.5	50	12.5	0	25
	SD 12	25	0	6.25	50	12.5	6.25	0	50
	SD 13	25	0	12.5	50	50	6.25	0	50
	SD 17	50	0	50	50	50	12.5	0	50
	SD 23	25	0	50	25	25	50	0	50
	SD 25	50	0	25	50	25	50	0	50
	SD 26	25	0	50	50	25	25	0	50
	SD 28	12.5	0	25	25	50	50	0	50
	SD 29	25	0	50	50	50	25	0	50
	SD 30	50	0	50	50	50	12.5	0	50
	SD 31	25	0	50	50	25	25	0	50
	SD 32	12.5	0	25	50	25	12.5	0	50
T. rubrum var velvety (velutinum)	SD 4	50	0	25	50	12.5	25	0	50
	SD 9	50	0	25	50	25	25	0	50
	SD 14	50	0	25	50	50	25	0	50

The high antifungal activity present in neem seed oil, 'tulsi' leaf and garlic scales is an important observation worth harnessing in preparation of herbal drug.

Earlier, Dutta *et al*[12]., have reported antifungal activity in water extracts of various parts of *Terminalia chebula, Punica granatum, Delonix regia* and *Emblica officicals* against spp of *Trichophyton* and *Microsporum.* Shahi *et. al.*,[18] reported antifungal activity in stored essential oils of some *Eucalyptus* spp. Against *Epidermophyton floccosum, Microsporum gypseum, M. nanum, Trichophyton mentagrophytes, T. rubrum* and *T. violaceum.*

The search for herbal sources of antifungal agents is a fast emerging area and holds great promise in providing safe antifungal drug free from side or adverse effects in man.

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References

1. Bulmer, G.S. (1979) *Introduction to Medical Mycology,* Year Book Medical Publishers, Chicago, p. 80.
2. Jacobs, P.H. (1990) *J. Am. Acad. Dermato.* **23 :** 549.
3. Maoz, M. & Neeman, I. (1998) *Lett. Appl. Microbiol.* **26 :** 61.
4. Torok, I. & stehlich, G. (1993) *Therapia Hungarica.* **41 :** 60.
5. Lopez-Gomez, S., Del Palacio, a., Van Gustem, J., Soledad Cuetara, M., Iglesias, L. & Rodriguez-Noriega, A. (1994) *Int. J. of Dermator.* **33 :** 743.
6. Rai, M.K. & Upadhyay, S. (1988) *Antibiot. Bull.* **30 :** 32.
7. Dikshit, A., Singh, A.K. & Dixit, S. N. (1981) J. *Antibact. Antifung. Agents.* **9 :** 9
8. Dikshit, A., Srivastava, O.P. & Husain, A. (1985) *J. Antibact, Antifung. Agento.* **13 :**57.
9. Dikshit, A. & Dixit, S.N. (1982) *Indian Perfumer.* **26 :** 216.
10. Singh, V.K. & Pandey, D.K. (1989) Hindustan, *Anttibiot. Bull.* **31 :** 32.
11. Palanichamy, S. & Nagarjan, S. (1990) *J Ethnopharmacol.* **29 :** 337.
12. Dutta, B.K., Rahman, I. & Das, T.K. (1998) *Mycoses.* **41 :** 535.

13. Summerbell. R & Kane, J (1999) in *Laboratory Handbook of Dermatophytes*. ed. Kane, J: Star Publishing Company, USA. p. 45.

14. Shadomy, S.A. & Pfaller, M.A. (1991) *Manual of Clinical Microbiology* eds. Balows, A., Hausler, W. J., Herrman, K., Isenberg, H.D. & Shadomy, H. J., 5th edn. Am. Soc. Microbiol, Washington, D.C.P. p. 1173.

15. Odds, F.C. (1992) Sabouraud ('s) agar. *J med Vet Mycol* **29 :** 355.

16. Hazen, K.C. (1998) *J. Am. Acad. Dermatol.* **38 (5, 3) :** S 37.

17. Hazen, K.C. (2000) Personal communication.

18. Shahi, S.K., Shukla, A.C. & Pandey, M.C. (1996) *Proceedings of the Ninetieth All India Botanical conference*. Vol. 50. p. 75.

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Effect of soil inoculation with *Glomus mosseae* at different P levels on flowering response of chrysanthemum

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Abstract

A pot experiment was conducted to study the response of chrysanthemum to *Glomus mosseae* at three levels of phosphorous i.e. 100, 75 and 50% of the recommended P. In general, plants inoculated with *Glomus mosseae* at 75% of the recommended P were taller, had more branches, maximum plant spread, and plant biomass as compared to the uninoculated plants. Mycorrhizal inoculation resulted in early flower bud initiation and early flowering. The flower number and flower yield per plant was enhanced when the plants were inoculated with the VAM fungus at 75% of the recommended P. It may be concluded that the best flowering can be obtained in the mycorrhizal plants at 75% of the recommended P and further, there is a possibility of reducing phosphatic fertilizer application by 25% through VAM inoculation in chrysanthemum.

(**Keywords :** chrysanthemum/*Glomus mosseae*/P fertilizer/VA mycorrhiza)

Introduction

Chrysanthemum is the most popular florists' flower. It is known as "Queen of East" and the most important cut flower cultivated world wide after carnation and rose. Chrysanthemum occupies pride of place both as a commercial flower crop and as a popular exhibition flower. For the production of good quality cut flowers, the major nutrients N, P and K must be supplied in adequate quantities. Phosphorous is a major element influencing growth and yield of flowers and P deficiency is known to retard flowering and decrease the number of flower buds[1].

Vesicular arbuscular mycorrhizal (VAM) fungi are ubiquitous group of soil microorganisms known to form symbiotic association with roots of economically important crop plants[2]. The beneficial effects of VAM fungi on crop plants is well documented[2]. The importance of this association is well recognised because of the key role of VAM fungi in enhancing the uptake of diffusion limited nutrients such as

P, Zn, Cu, etc., especially in soils of low nutrient status. Inoculaiton with VAM fungus causes greater uptake of P by their expanded network of hyphae[3,4]. In the present study symbiotic response of chrysanthemum to inoculation with the VAM fungus *Glomus mosseae* at three levels of phosphorus, 100, 75, and 50% of the recommended P was studied and compared with the uninoculated plants given 100% P in order to know the possibility of reducing the application of phosphatic fertilizer through VAM inoculation.

Materials and Methods

Rooted cuttings of *Dendrathma grandiflora* (*Chrysanthemum morifolium* Ramat.) var. 'Red Gold' were planted in potting mix consisting of sand : soil : compost in 1:2:1 proportion in 19 cm diameter pots of 3.5 kg capacity. The potting mixture used was deficient in phosphorus (3mg/Kg available P; NH_4F + HCl extraction) with pH 5.7 and an indigenous VAM population of 30 spores/ 50 ml soil. There were 4 treatments with 13 replications and pots were arranged randomly on the glasshouse benches. The treatment details are as follows :

UP 100 = Uninoculated control, 100% of the recommended P without VAM.

IP 100 = Inoculated, 100% of the recommended P.

IP 75 = Inoculated, 75% of the recommended P.

IP 50 = Inoculated, 50% of the recommended P.

The NPK fertilizers were supplied in the form of urea, single super phosphate, and muriate of potash. N and K fertilizers were supplied at the recommended rate (125 Kg N and 100 Kg K/ha) uniformly for all the pots while P fertilizer was added at different levels based on the treatments. The recommended level of P is 150 KgP/ha. The fertilizers were thoroughly mixed with the planting medium and the pots were watered till field capacity. Mycorrhizal inoculum consisted of infected roots and soil from a pot culture of Rhodes grass, which had been infected with *Glomus mosseae* and grown for 90 days. The inoculum contained hyphae, vesicles, arbuscules and chlamydospores of *G. mosseae*. The inoculum containing 150 spores/50 ml was placed in planting hole at the rate of 25 g/plant. One plant per pot was maintained.

The plants were watered whenever necessary. Pinching (removal of terminal shoot bud to promote branching) was done during the seventh week after transplant. Observations on plant growth parameters like plant height, number of branches, plant spread, and dry matter production were taken one month after planting and the subsequent observations were taken at 15 days interval till harvest on 120 days after

planting (DAP). However, only the data on 120 DAP is presented in this paper. Observations on the time taken for bud initiation and 50 per cent flowering were recorded. The flowers were harvested when the central whorl of petals were found in opened condition. Thus, two harvests were done; first harvest at 105 DAP and second harvest at 120 DAP. Observations on number of flowers per plant, flower size, stalk length and flower yield were recorded. Vase life of cut flowers, harvested at 120 DAP, was studied in 2% sucrose solution at room temperature (20 to 27 °C).

Extramatricular chlamydospores produced by the VAM fungus in soil was estimated by wet sieving and decanting method[5]. The extent of VAM colonization in roots was determined by grid line intersect method[6] after staining the roots with acid fuchsin[7]. The shoot (leaves + stems) and root P concentration was estimated by vanadomolybdate phosphoric yellow colour method[8].

The data was subjected to statistical analysis suitable to RCBD and the treatment means were separated by Duncan's Multiple Range Test (DMRT)[9].

Results and Discussion

Maximum plant height, number of branches and plant spread was observed in plants inoculated with VAM fungus and given 75% of the recommended P though it was statistically on par with inoculated plants given 100% P. Inoculated plants given 50% P had significantly lower plant height, number of branches and plant spread compared to all other treatments (Table 1). Mycorrhizal inoculation increasing plant height and spread has been reported in marigold[10], asters and salvias[11] and zinnia[12]. This influence of VAM fungi on growth parameters may be due to increased P uptake, which might have caused cell elongation and multiplication[13].

Maximum plant biomass, maximum shoot (leaves + stems) and root biomass was observed in plants inoculated with VAM fungus given 75% of the recommended P (Table 1). Similar observations were made with *Lilium regale*[14] and with *Chrysanthemum morifolium*[15]. Inculation with VAM fungus in known to increase the uptake of micronutrients like Cu, Mn, Fe and Zn in addition to P uptake[16,17]. VAM fungus is also known to enhace water uptake in plants[18]. These beneficial effects of VAM fungus might have contributed for maximum dry matter production in the inoculated plants. Mycorrhizal plants gwown in the presence of 75% of the recommended P had maximum shoot and root P content as compared to the other treatments (Table 1) demonstrating the ability of this fungus to efficiently translocate phosphorus from soil to the host through their ramified hyphae, which contributes for increased surface area of absorption.

Table 1– Effect of soil inoculation with *Glomus mosseae* at different P levels on plant height, number of branches, plant spread, biomass and P content of chrysanthemum

Treatment	Plant height (cm)	Plant spread (cm)	Number of branches/ plant	Biomass (g/plant)		P content (mg plant)	
				Shoot	Root	Shoot	Root
UP 100	57.92a	49.62a	33.38b	99.85b	36.03ab	119.82d	93.67b
IP 100	58.38a	48.73a	38.15a	100.85b	31.96b	161.36b	86.29cb
IP 75	61.15a	49.92a	38.69a	118.70a	44.59a	379.84a	147.14a
IP 50	51.85b	37.15b	30.92b	78.85c	29.20b	122.19c	75.92d

Means followed by the same superscript within each column do not differ significantly at P = 0.05.

UP 100 = Uninoculated control, 100% P without VAM.

IP 100 = Inoculated, 100% of the recommended P.

IP 75 = Inoculated, 75% of the recommended P.

IP 50 = Inoculated, 50% of the recommended P.

Inoculation with the VAM fungus hastened flower bud initiation stimulated early flowering and resulted in increased flower number and flower yield (Table 2). Time taken for flower bud initiation was maximum in uninoculated plants (76 days) while it was least in the inoculated plants grown in the presence of 75% of the recommended P (73 days). A similar trend was observed with respect to days taken for 50% flowering. Plants inoculated with VAM fungus at 75% of the recommended P took 118 days for 50% flowering compared to 137 days taken by the uninoculated plants. Maximum number of flowers (139 per plant) were harvested from plants inoculated with VAM fungus at 75% of the recommended P level while number of flowers per plant in uninoculated plants given 100% P was only 105. Flower yield per plant was also maximum in plants inoculated with the VAM fungus at 75% of the recommended P which corresponded to 23.32% increase in yield over the uninoculated control plants given 100% P. Flower yield is the manifestation of yield contributing characters like number of flowers per plant and flower size. Increase in flower number and flower yield per plant when inoculated with VAM fungus as compared to uninoculated plants has been observed in *Juniperus chinesis*[19] and in *Chrysanthemum frutescens*[20]. The differences in yield components may be attributed to physiological characters, both in vegetative and reproductive stages of growth because of enhanced nutrient translocation by VAM fungi[2].

Inoculation with VAM fungi had a significant effect on flower characteristics like flower diameter and stalk length (Table 2). Maximum flower diameter (5.25 cm) and stalk length (11.72 cm) was observed in plants inoculated with VAM fungus at 75% of the recommended P, the increases being 5.4% and 6.73% with respect to flower diameter and stalk length respectively over the uninoculated plants given 100%P. Flowers from plants inoculated with the VAM fungus given 75% of the recommended P had maximum vase life compared to flowers from other treatments. Flowers from inoculated plants given 75% of the recommended P lasted for 5 days more in 2% sucrose solution, as compared to flowers obtained from uninoculated plants, given 100% P. The increased vase life of flowers from mycorrhizal plants may be due to greater development of water conducting tissues in mycorrhizal plants than in non-mycorrhizal plants[21,22].

Table 2– Effect of soil inoculation with *Glomus mosseae* at different P levels on flowering response of chrysanthemum

Treatment	Days taken for bud initiation	Days taken for 50% flowering	Number of flowers/ plants	Flower yield/ plant (g)	Flower stalk diameter (cm)	Stalk length (cm)	Dry wt. (g)	Flower longevity (days)
UP 100	76.23a	137.54a	105.85b	124.92b	4.98b	10.68b	27.84	9c
IP 100	73.69c	127.08c	123.54b	164.06a	5023a	11.28b	27.95	10c
IP 75	73.69c	118.54d	138.69a	178.72a	5.25a	11.72a	29.34	14a
IP 50	75.31b	128.85b	99.00b	123.35b	5.00b	10.45b	24.11	12b
							NS	

Means followed by the same superscript within each column do not differ significantly at P =0.05.

Legend as in Table 1.

Inoculation with *Glomus mosseae* markedly increased the level of VAM colonization in roots of chrysanthemum at all the three P levels as compared to the uninoculated plants (Table 3). However, per cent colonization was significantly higher in mycorrhizal plants given 75% of the recommended P. The extramatricular chlamydospore numbers in the root zone soil also followed a similar trend. This suggests that the number of infective propagules of the indigenous mycorrhizal fungi in the growth medium used in the present study is not only low but also ineffective.

Table 3 – Effect of soil inoculation with *Glomus mosseae* at different P levels on spore numbers in the root zone soil and per cent mycorrhizal root colonization of chrysanthemum

Treatment	Spore count/50 ml. soil	VAM colonization (%)
UP 100	182.92d	46.36d
IP 100	227.70c	78.19c
IP 75	325.54a	94.70a
IP 50	266.00d	85.02b

Means followed by the same superscript within each column do not differ significantly at P =0.05.

Legend as in Table 1.

The results of the present study clearly indicate that chrysanthemum responds well to inoculation with *Glomus mosseae*. By plant growth characteristics and flower yield, it can be stated that soil inoculation with *Glomus mosseae* at 75% of the recommended P is beneficial. It can be concluded that through mycorrhizal inoculation, P fertilizer application can be reduced by 25%.

References

1. Tinker, P.B.H. (1975) in *Endomycorrhizas*, eds. Sanders, F.E., Mosse, B. and Tinker, P.B.H., Academic Press, London.
2. Bagyaraj, D.J. &Varma, A., (1995) in *Advances in Microbial Ecology*, ed. Jones, J.G., Academic Press, London.
3. Bolon, N.S. (1991) *Plant Soil*, **134** : 189.
4. Sreenivasa, M.N. (1992) *Scientia Horticulturae*, **50** : 53.
5. Gerdemann, J.W. & Nicolson, T.H. (1963) *Trans. Br. Mycol. Soc.* **46** : 235.
6. Giovannetti, J.W. & Mosse, B. (1980) *New Phytol.* **84** : 489.
7. Kormanik, P.P., Bryan, W.C. & Schultz, R.C. (1980) *Can. J. Microbiol.***26** : 536.
8. Jackson, M.L. (1973) *Soil Chemical Analysis*, Prentice Hall, New Delhi.
9. Little, T.M. & Hills, F.J. (1978) *Mean Separation in Agricultural Experimentation*, John Willey and Sons, New York.
10. Bagyaraj, K.J. & Powell, C.L. (1985) *N.Z.J. Agril. Res.* **28** : 169.
11. Kale R.D., Kubra Bano, Sreenivasa, M.N. & Bagyaraj, D.J. (1987) *South Indian Hort.* **35** : 433.

12. Cazares, E. & Smith, J.E. (1996) *Mycorrhiza*, **6 :** 61.
13. Black, C.A. (1965) in *Methods of Soil Analysis II*, ed. Page, A.L. Madison, Wisconsin, USA.
14. Vanderploeg, J.L., Lightly, R.W. & Sasser, M. (1974) *Hort. Sci.* **9 :** 383.
15. Johnson, C.R., Menge, J.A. & Johnson, E.L.V. (1982) *Scientia Horticulturae.* **7 :** 265.
16. Cooper, K.M. & Tinker, P.B. (1978) *New Phytol.* **81 :** 43.
17. Krishna, K.R. & Bagyaraj, D.J. (1985) in *Proceedings of 6th NACOM*, ed. Molina, R., Forest Research Laboratory, Orgon State University, Corvalis.
18. Allen, M.R., Moore, T.S. & Christensen, M. (1982) *Can. J. Bot.* **58 :** 371.
19. Ronkadori, R.W. & Pokorny, F.A. (1982) *Hort. Science.* **17 :** 917.
20. Backhans, G.F. (1982) in *Abstracts 21st Int. Hort. Congress.* Hamburg.
21. Chang, D.C.N. (1992) *Sci. Agric.* **40 :** 45.
22. Wen, C.L. (1991) *MS Thesis*, Dept. of Hort., National Taiwan Univ.

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Production of cellulases by three isolates of *Syncephalastrum racemosum* (Cohn.) Schroet in relation to pathogenicity

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Abstract

Production of cellulases (C_1 & C_x) by three isolates of *Syncephalastrum racemosum* (Cohn) Schroet. isolated from lemon, orange and mosambi fruits under different cultural conditions was investigated. In general mosambi isolate was poor producer of cellulases (C_1 & C_x), while orange isolate was efficient in this task. D-galactose was the best carbon source for induction C_x enzyme in all the three isolates of *S. racemosum* under investigation. However the favourable carbon source for C_1 production varied with the isolate of *S. racemosum*. Cellulases of all the three isolates were adaptive.

(**Keywords** : cellulases/*Syncephalastrum racemosum*/carbon/nitrogen sources)

Introduction

Cellulases produced by pathogenic fungi play a significant role either directly by degrading cellulose cell wall or indirectly by releasing nutrients from disrupted cells. Goyal and Mehrotra[1] Ward and Moo-Young[2] and Bhalla and Joshi[3] have observed a positive correlation between cellulolytic activity and disease development. Cellulases are also reported to play an important role in competitive saprophytic survival of the pathogen[4]. *S. racemosum* was serious and common pathogen[5] of lemon, orange and mosambi fruits. Hence, in the present investigations, potentiality of three isolates of *S. racemosum* to produce cellulases in relation to pathogenicity was studied.

Materials and Methods

Monosporic cultures of three isolates of *Syncephalastrum racemosum* (Cohn) Schroet. isolated from lemon (*C. media var. medica* L.), orange (*C. reticulata*, Blanko) and mosambi (*C. sinesis* Osbeck) fruits and maintained on Asthana and Hawker's medium A, were employed in the present studies. The following five synthetic media were selected.

A. Asthana and hawker's medium 'A' (Glocose 10g, KNO_3 3.8g, KH_2PO_4 1.75g, $MgSo_4$ 0.75g and 1 litre of water)

B. Richard's medium (Sucrose 50g, KNO_3 10g, KH_2PO_4 5g, $MgSO_4$ 2.5g, $FeCl_2$ 0.02g and distilled water 1000 ml.)

C. Modified Czapek's medium (Sucrose 10g, $NaNo_3$ 3.0g, KH_2PO_4 1.0g, $MgSO_4$ 0.5g, KCl_2 0.5g and distilled water 1000ml).

D. Modified Czapek's medium + o.5% CMC.

E. Singh and Wood medium (H_2PO_4 1.0g, Asparagine 4.0g, $MgSO_4$ 0.5g Glucose 5g, Pectin 10g and distilled water 1000ml)

The fungi were grown in 100ml Erlenmayer conical flasks containing 25ml of different synthetic medium at room temperature (27 ± 2^0C) for 12 days. At the end of 4,8 and 12 days of incubation, a set of flasks were harvested in Whatman filter paper for determination of growth[6]. The culture filtrates were employed as enzyme sample after centrigfugation at 1800 X g for 30 minutes and dialysed against glass distilled water.

The cellobiohydralase (EC 3.2.1.91; C_1) activity was determined by DNS method as suggested by Miller[7]. The endo-gluconase (EC 3.2.1.4; Cx) activity was assayed by viscosity loss as suggested by Reese et al.[8].

Influence of different carbon and nitrogen sources on cellulase secretion by 3 isolates of *S. racemosum* was also studied by substituting the glucose and potassium nitrate of Asthana and Hawker's medium 'A' with different carbon and nitrogen sources so as to supply equivalent amount of carbon (4000 mg/L) and nitrogen (483 mg/L) and nitrogen (483 mg/L) respectively.

Results and Discussion

Medium D supported maximum production of cellulase (Cx) by all the three isolates under study (Table-1). Medium E followed by A were the next preferred substrates. On medium A, the lemon isolate produced increasing amount of cellulase till the end of incubation period, while other two isolates could secrete cellulase up to 8^{th} day of incubation. Increased amount of cellulase in CMC containing media suggests the adaptive nature of cellulase of present fungi. Similarly Hasija and Batra[9] and Sharma et al.[10] have reported the adaptive nature of cellulase of fungi studied by them. In general orange isolate was efficient in the production of cellulase, while mosambi isolate was poor in this task.

Table 1– Production of cellulases (C_x* and C_1*), mycelial growth and pH changes during the growth of three isolates of *S. racemosum* on different synthetic media.

Medium		Lemon isolate			Orange isolate			Mosambi isolate		
		4	8	12	4	8	12	4	8	12
Asthana and Hawker's	Dry Wt. (in mg)	44.0	46.0	53.0	51.0	53.0	170.0	49.0	58.0	78.0
medium[4](A)	PH	6.0	6.5	6.0	6.5	6.5	7.0	6.5	7.0	7.6
Endogluconase	(Cx)	8.69	22.22	26.66	5.33	11.10	-	6.89	13.33	-
Cellobiohydralase	(C_1)	-	0.11	0.14	0.11	0.51	0.19	-	0.12	-
Richard's medium (B)	Dry Wt. (in mg)	139.0	206.0	216.0	105.0	101.0	86.0	139.0	162.0	192.0
	PH	6.0	6.0	7.0	6.0	6.5	6.5	5.5	7.5	7.5
Endogluconase	(Cx)	-	22.2	13.5	-	33.3	11.1	-	-	16.6
Cellobiohydralase	(C_1)	.60	0.13	0.15	0.54	0.52	0.36	1.02	0.12	-
Modified Czapek's	Dry Wt. (in mg)	84.0	111.0	75.0	87.0	131.0	188.0	26.0	138.0	145.0
medium (C)	PH	6.0	7.0	7.5	6.0	6.5	7.5	6.0	7.0	7.5
Endogluconase	(Cx)	-	18.28	20.0	20.0	22.2	-	12.1	18.5	6.99
Cellobiohydralase	(C_1)	0.140	0.17	0.13	0.13	0.17	0.13	0.24	0.16	-
Modified Czapek's	Dry Wt. (in mg)	105.0	120.0	111.0	36.0	152.0	187.0	94.0	114.0	98.0
Medium + 0.5% CMC	PH	6.0	7.0	7.5	6.0	6.5	7.5	6.0	7.0	7.5
Endogluconase	(Cx)	7.14	25.0	13.3	13.3	33.3	25.0	6.3	8.3	20.0
Cellobiohydralase	(C_1)	0.11	0.33	0.31	-	0.21	0.13	-	0.35	-
Singh and Wood's	Dry Wt. (in mg)	91.0	176.0	169.0	91.0	113.0	132.0	107.0	112.0	175.0
Medium (E)	PH	4.0	8.0	7.5	4.5	8.5	7.5	3.5	7.5	7.5
Endogluconase	(Cx)	22.0	28.9	27.4	10.1	29.6	14.3	10.1	18.51	17.7
Cellobiohydralase	(C_1)	0.13	0.15	-	014	018	012	0.25	0.21	0.12

* Expressed in relative viscometric units (RVU)

+ Expressed as the increase in mg of reducing sugars (as glucose/ml) liberated in 6 hrs of incubation.

Though all the three isolates, secreted cellobiohydralase in one or other medium at one or other incubation period, they differed significantly in the degree of production. With a few exceptions 8th day incubation was optimum for enzyme production.

Lemon isolate attained maximum vegetative growth in medium B. Medium E was the next choice. Medium A was the poor substratum for the growth of this isolate. Orange isolate preferred medium C and D followed by medium A. On the other hand, medium B supported maximum growth of mosambi isolate, while medium A the least. With a few exceptions, all the three isolates showed continuous increase in growth till the end of incubation period tried.

Prolific production of cellulases by range isolate of *S. recemosum* may be one of the attributes for its virulence. In general pH of the medium drifted towards alkaline side. The pH drift was significant in medium E. No correlation could be observed between the enzyme production and pH of the medium.

Galactose followed by sorbitol supported maximum Cx production by lemon isolate, while L-sorbose induced least amount of Cx (fig. 1). D-glactose and L-sorbose were good carbon sources for induction of cellulase (Cx) in orange isolate. Hasija and Batra[9] have reported enhanced Cx activity in the presence of L-sorbose. Mosambi isolate secreted good amount of cellulase on starch and D-galactose, while on D-fructose and sucrose, secretion of cellulase was very meagre.

Lemon isolate failed to secrete C_1 on D-galactose, lactose and sorbitol, while it secreted maximum C_1 during its growth on starch. Orange isolate failed to secrete C_1 enzyme on sucrose but secreted C_1 enzyme on glucose medium. Mosambi isolate failed to secrete C_1 on sucrose and sorbitol but it secreted maximum C_1 on L-sorbose followed by D-galactose.

Lemon isolate opted glutamine followed by DL-aspartic Acid and L-methoinine for the production of cellulase (Cx) (Fig. 2). L-glycine and sodium nitrate were poor in the induction of cellulase. Peptone and L-histidine were efficient inducers of cellulases in organe isolate. DL-aspartic acid followed by potassium nitrate were poor substrates for the secretion of this enzyme. Though mosambi isolate secreted Cx enzyme in all the nitrogen media, DL-aspartic acid and L-glutamine were poor in the induction of Cx enzyme. Peptone and caesin were responsible for maximum production of cellulase (Cx).

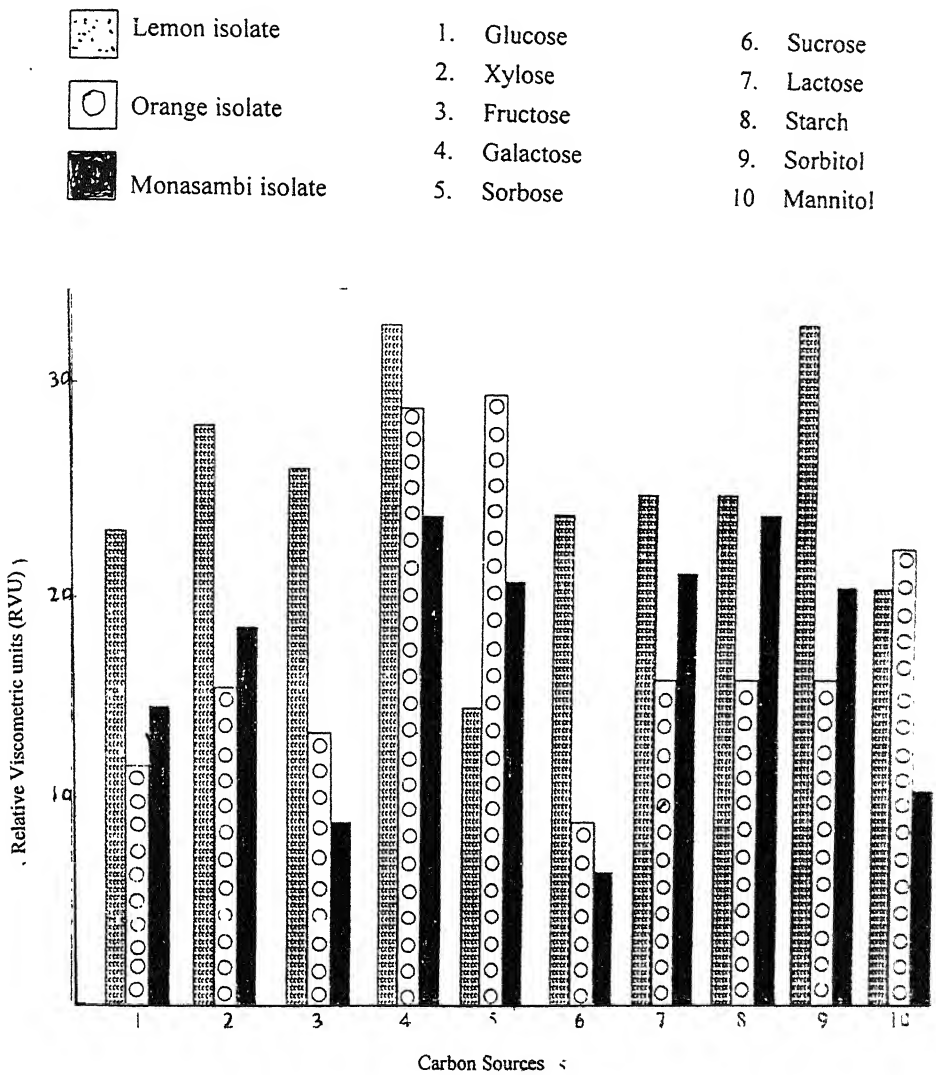

Fig 1– Effect of different carbon sources on cellulase (C_x) production by three isolates of *S. racemosum*

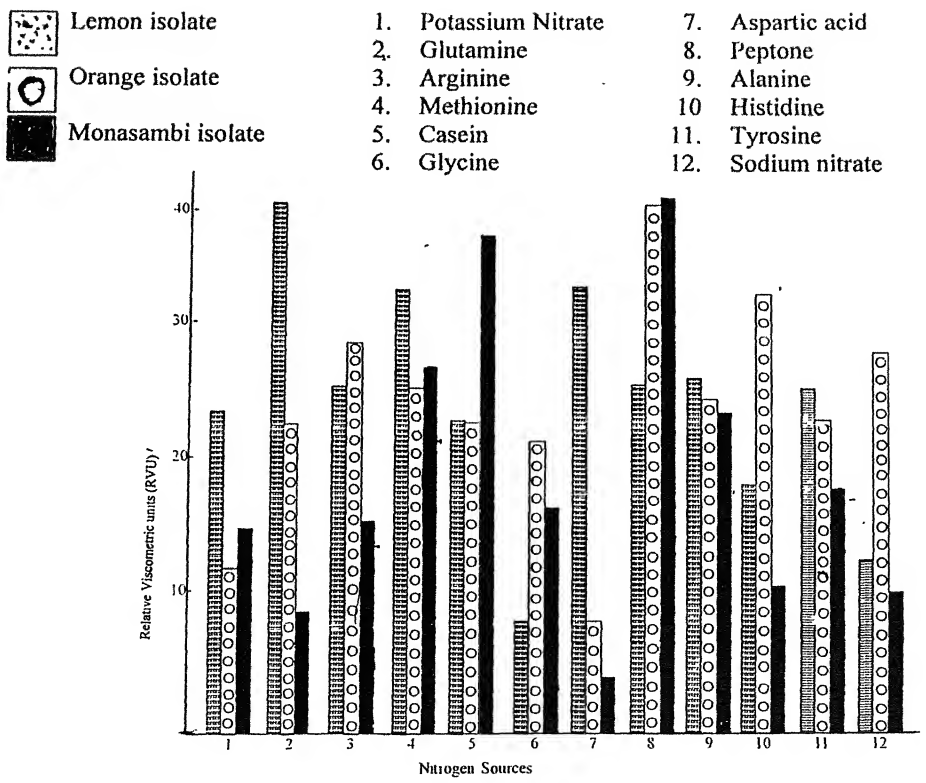

Fig. 2– Effect of different nitrogen sources on cellulase (C_x) production by three isolates of *S. recemosum*.

Sodium nitrate, L-glycine and glutamine failed to induce C_1 enzyme in lemon isolate, while caesin induced maximum C_1 L-methionine and L-glycine the orange isolate for production of C_1 enzyme, while potassium nitrate induced maximum C_1 enzyme. Mosambi isolate failed to secrete C_1 enzyme, while potassium nitrate induced maximum C_1 enzyme. Mosambi isolate failed to secrete C_1 on L-arginine, L-glycine, DL-aspartic acid and peptone, while, L-glutamine and DL-alanine were responsible for maximum C_1 anzyme production. Potassium nitrate supported least activity of C_1 enzyme.

Similar to present observatins, Narania and Reddy[11], Reddy and Reddy[12], and Chary and Reddy[13] have reported the specificity of fungi for nitrogen source in the induction of cellulases studied by them. Further, severity of orange fruit-rot caused by *S. racemosum* may be partly due to its potentiality of secreting cellulases.

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References

1. Goyal, M.K. & Mehrotra, R.S. (1980) *Acta Bot Indica* **8(1)** : 74.
2. Ward O.P. & Moo-Young (1989) *CRC Critical Reviews* in Biotechnology **8(4)** : 237.
3. Tekchand Bhalla & Monica Joshi (1993) *Indian Journal of Microbiology* **33(4)** : 353.
4. Ghewande, M.P. & Deshpande, K.B. (1979) *Proc. Ind. Nat. Sci. Acad.* **45B(1)** : 29.
5. Jagadish Babu, K.Lakshminarayana, P & Reddy, S.M. (1982) *Curr. Sci.* **51(18)** : 892.
6. Reddy, S.M. & Ananthan, K (1987) *Zentralbl Mikrobiol.* **142** : 179.
7. Miller, G.L. (1959) *Analytic Chem.* **31** : 426.
8. Reese, E.T. Sieu, R.B. & Levinson, H.S. (1950) *J. Bacteriol* **59** : 485.
9. Hasija, S.K. & Batra (1982) *Indian Phytopath.* 35 : 384.
10. Sharma, S.G., Ramnarayan & Sangamlal (1984) *Indian Phytopath* **37** : 534.
11. Narania, Kantialal & Reddy. S.M. (1984) *Proc. Nat. Acad. Sci India* **54(B)** : 88.
12. Reddy, A.S. & Reddy, S.M. (1985) *J. Indian. Bot. Soc.* **64** : 377.
13. Chary, S.J. & Reddy, S.M. (1985) *Proc. Nat. Acad Sci India.* **55(B)** : 326.

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A typical yield response of fibre crops to fertilizer use under rice necrosis mosaic virus inoculation

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Abstract

Fibre crops namely, *Corchorus olitorius* L. cv. JRO-632, *C. capsularis* L. cv. JRC-212, *Hibiscus sabdariffa* L. cv. HS-4288 and *H. cannabinus* L. cv. HC-583 were mechanically inoculated with the sap obtained from the leaves of rice plants infected with rice necrosis mosaic virus. Inoculation was done at three different ages of crop grown with three different levels of fertilizers under field condtion and their fibre productions were compared. In case of *C. olitorius, H. sabdariffa* and *H. cannabinus,* plants inoculated at 10 to 40 days of crop grown with half of the recommended dose of fertilizers produced highest fibre yield. While in case of *C. capsularis,* plants inoculated at 10 days of crop grown with full dose of fertilizers produced maximum yield of fibre. Fibre crops inoculated with sap obtained from leaves of healthy rice plants did not show any such effect on any of the fertilizer levels and crop ages used. Virus inoculated plants, in general, showed higher vigour than in control ones without any visible virus symptom in them.

(**Keywords :** rice necrosis mosaic virus/ fertilizer/growth promotion/fibre crop)

Rice necrosis mosaic virus (RNMV) stunts the growth and reduces yield in rice plant[1-3] whereas some fibre crops, artificially inoculated with RNMV, grew faster, produced higher bio-mass and increased yield within a stipulated period. Definite association of RNMV with such growth promotion in fibre crops was observed through electron microscopic and serological studies[4,5]. To get an idea about the role of fertilizer requirement in such interactions with different fibre crops belonging to different families, the present investigation was carried out as per standard practices[6] under field condition in randomized block design using various levels of fertilizers for two different years (1985 & 1986).

Rice plants infected with RNMV, kept within screened nethouse served as sources of inoculum for inoculation. Fibre crops like *Corchorus olitorius* cv. JRO-632, *C. capsularis* cv. JRC-212 belonging to family Tiliaceae and *Hibiscus sabdariffa* cv. HS-4288, *H. cannabinus* cv. HC-583 belonging to family Malvaceae served as experimental plants. Fertilizers used in three different levels namely were N_0 P_0 K_0, N_{20} P_{10} K_{10} and N_{40} P_{20} K_{20} (recommended dose) for *C. olitorius* and N_0 P_0 K_0, N_{30} P_{15} K_{15}, N_{60} P_{30} K_{30} (recommended dose) for *C. capsularis, H. sabdariffa* and *H. cannabinus*. Nitrogenous fertilizers were used in two split doses whereas P&K used as basal. Three different stages of crop growth namely 10, 40 and 70 days after emergence were considered for mechanical inoculation on 4th leaf (from top) with the virus inoculum. The experimental

plants (including control ones) were allowed to grow up to harvest (120 days for *Corchorus* group and 140 days for *Hibiscus* group). Data obtained from 50 plants in each replicated plot grown in two years were analyzed any presented.

Plants inoculated with RNMV showed more juvenility and increase in pant height with varying degrees in different crops tested over respective controls. None of the inoculated plants exhibited any visible virus symptom on them. The effect of virus inoculation was greatest from early inoculation, declining as inoculation was later.

C. olitorius plants, inoculated with the virus at 10 days of age, grown with half of the recommended dose of fertilizer produced highest dry fibre yield over respective control whereas in case of *C. capsularis*, plants inoculated at the same age but grown with full recommended dose of fertilizer produced maximum yield (Table 1). In case of *H. sabdariffa* and *H. cannabinus*, plants inoculated at 10 and 40 days of crop growth respectively and grown with half of the recommended dose of fertilizer produced highest fibre yield as compared with those of control (Table 2).

The study thus showed a striking ability of RNMV in plant growth promotion and highlighted a beneficial nature of host-virus interaction. In case of *olitorious* Jute, fertilizer requirement is not very high whereas *capsularis* Jute and plants belonging to *Hibiscus* group are heavy feeders. In such a situation, optimum functioning of different host metabolic processes for optimum output was perhaps conditioned by RNMV. From commercial point of view, the *Corchorus* group of plants are harvested within 120 days of growth for fibre and their production-oriented metabolism also started early[6]. But plants belonging to *Hibiscus* group take longer period of around 140 days for production of commercially viable fibre and hence more amount of fertilizers are needed for their development *in situ*. Furthermore, RNMV behaved differently in two crops, under study, of same *Hibiscus* genus with regard to fibre production; inoculation at 10 days in case of *H. sabdariffa* and 40 days in case of *H. cannabinus* produced maximum fibre yield. So with the availability of suitable environment *in situ* at 10-40 days of plant growth, RNMV after entry into the host possibly triggered the synthesis of growth hormones associated with higher growth and yield[7]. Reasons behind such diversified effect are still obscure and warrants further study.

Table 1– Yield of *Corchorus* group of fibre plants inoculated with rice necrosis mosaic virus at different age groups with different fertilizer levels in different years (pooled)

Plant inoculation (Days after emergence)	Dry fibre yield (g)							
	Corchorus olitorious (cv. JRO-632) Fertilizer levels (kg / ha)				*Corchorus capsularis* (cv. JRC-212) Fertilizer levels (kg / ha)			
	$N_0P_0K_{10}$	$N_{20}P_{10}K_{10}$	$N_{40}P_{20}K_{20}$	Mean	$N_0P_0K_{10}$	$N_{20}P_{10}K_{10}$	$N_{40}P_{20}K_{20}$	Mean
10	420.38	536.13	491.25	491.59	243.50	267.38	344.13	285.00
40	429.25	483.13	482.13	464.84	232.88	242.13	288.50	254.50
70	452.50	447.63	436.63	445.59	205.13	285.75	273.75	254.88
Control (uninfected)	348.25	405.25	450.25	401.25	219.13	221.75	260.88	233.72
Mean	412.60	474.79	465.07	–	225.16	254.25	291.82	–

S.E. for Inoculation (I)	: 18.59	: 16.53
" " Fertilizer (F)	: 16.10	: 14.32
" " I x F	: 32.19	: 28.64

Table 2 - Yield of *Hibiscus* group of fibre plants inoculated with rice necrosis mosaic virus at different ages grown under different fertilizer levels in different years (pooled)

Plant inoculation (Days after emergence)	Dry fibre yield (g)							
	Hibiscus sabdariffa (cv. HS-4288) Fertilizer levels (kg / ha)				*Hibiscus cannabinus* (cv. HC-583) Fertilizer levels (kg / ha)			
	$N_0P_0K_0$	$N_{30}P_{15}K_{15}$	$N_{60}P_{30}K_{30}$	Mean	$N_0P_0K_0$	$N_{30}P_{15}K_{15}$	$N_{60}P_{30}K_{30}$	Mean
10	450.13	562.83	581.50	531.42	491.88	608.25	566.38	555.67
40	457.50	518.50	561.25	512.42	426.50	653.25	541.25	540.50
70	449.63	479.88	510.00	429.84	519.13	569.50	556.38	548.34
Control (uninfected)	387.88	417.63	483.38	429.63	406.50	466.25	517.00	463.25
Mean	436.29	494.66	534.03	-	461.00	574.56	545.25	-
S.E. for Inoculation (I)		: 24.91				: 21.03		
" " Fertilizer (F)		: 21.57				: 18.21		
" " I x F		: 43.14				: 36.42		

Enhancement in tillering and yield of some plants belonging to monocot is reported to be possible by using a microbe like *Bacillus subtilis* A13 in soil, infested with *Rhizoctonia, Pythium* and *Fusarium* and such effect is explained as siderophore activity[8,9]. But the present investigation shows the direct effect of RNMV in increasing yield. It is imminent with the present study that possibly an unusual type of either symbiotic or pseudosymbiotic relationship between the virus and the host had developed by which both the organisms were benefitted because in concurrence with plant growth promotion virus also multiplied inside the host and as a result more harvest was obtained. In Jute-rice rotation chances of infection of succeeding rice crop by this virus is very remote due to phyllogenetic differences and host specificity of fungal vector of this virus[5].

The present investigation, therefore, offers a unique technology wherein a virus plays a significant role in economizing the cost of cultivation by reducing the fertilizer requirement and saving the soil from further erosion through fertilizer use.

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References

1. Ghosh, S.K. (1980) *Proc. Indian Acad Sci.* (Plant Science) **89** : 291.
2. Fujii, S. (1967) *Shokubutsu Boeki* **21** : 188.
3. Invouye, T. & Fujii, S. (1977) *Rice necrosis mosaic : Description of Plant Viruses*, Ser no. 172, , Commonwealth Mycol. Instt. Surrey, England, p. 4.
4. Ghosh, S.K. (1982) *Planta* **155** : 193.
5. Ghosh, S.K. (1985) *J. agric. Sci.*, **105** : 141.
6. Kundu, B.C., Basak, K.C. & Sarcar, P.B. (1959) *Jute in India*, a monograph, Central Jute Comm. Publ., Calcutta.
7. Ghosh, S.K. (1995) *Int. J. Trop. Pl. Dis.* **13:** 221.
8. Baker, K.F. & Cook, RlJ. (1974) *Biological Control of Plant Pathogens*, W.H. Freeman & C., San Francisco, USA.
9. Suslow, T.V. & Schroth, M.N. (1982) *Phytopathology* **72** : 115.

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